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A STUDY OF THE FORM AND GROWTH OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN[1]

CURTIS L. NEWCOMBE, FRANK CAMPBELL AND ALLEN M. ECKSTINE

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Studies of the linear dimensions of *Callinectes sapidus* Rathbun have defined certain growth characteristics by indicating the general trend and the magnitude of change in form accompanying increase in size (Gray and Newcombe, '38a). Additional observations, made at the Chesapeake Biological Laboratory during the period 1935-1938 and at the Virginia Fisheries Laboratory during 1940-1944, provided a better understanding of the moulting behavior of this important edible crab (Gray and Newcombe, '38b; Lochhead, Lochhead and Newcombe, '42; Lochhead and Newcombe, '42; Sandoz and Rogers, '44; and Newcombe, Sandoz and Rogers-Talbert, '49).

Knowledge of the natural growth processes of this decapod contributes one more link to the chain of evidence required to reveal the nature of form and structural change in the arthropod type. Constancy of body proportions together with range and magnitude of the constant differential growth ratios of body parts in relation to the whole body are species characteristics of systematic value in Arthropods. Huxley ('27) analyzed data on Lucanids and demonstrated "that the various 'high' and 'low' forms which have been distinguished in these species have no valid taxonomic basis, but form one continuous series, the 'form' merely being a function of the total absolute size". We are interested in the form changes that may characterize species of *Callinectes* since there is a close resem-

[1]This study was conducted at the Virginia Fisheries Laboratory of the College of William and Mary and the Commission of Fisheries of Virginia at Yorktown and Williamsburg during the summer of 1946. The data have been analyzed and prepared for publication at and with the aid of the Cranbrook Institute of Science, Bloomfield Hills, Michigan.

blance between certain of the species, and also because modifications in body form and size are believed to accompany major differences in habitats occupied by the same species.

Attention is given to further observations on linear dimensions and, for perhaps the first time, weight characteristics of the body and of certain of its component parts have been studied experimentally.

Changes in body form accompanying increase in size are readily described from external linear measurements. Significant sex differences that have been demonstrated in several species are reflected in changing growth coefficients (here designated as b in the allometric equation $Y = aX^b$). An explanation of the degree of change must, in some instances at least, await an understanding of the growth characteristics of the internal parts of the body, each of which presumably has one or more growth coefficients relative to the whole body. In the case of a swimming form, such as the Blue Crab, increase in weight may have its limitations in so far as the survival of the species is concerned; hence the ratio of muscle development to exoskeletal weight and form is of prime importance. The keen competition for survival places a premium on motility which is largely a function of muscular development, body form, and weight.

Fresh crab meat is sold in three particular grades known as "lump" meat, the muscle tissue which moves the fifth pereiopod and known commercially as "back fin"; "flake" meat, the proximal muscles controlling the second, third and fourth pereiopods; and "claw" meat, the large muscle of the cheliped. Weights have been taken of these meats together with the remaining meats of the pereiopods, the visceral mass and the exoskeleton, and in the females with an egg mass or "sponge", the weight of the "sponge" has also been measured.

We are greatly indebted to Mr. O. R. Mills of Seaford, Virginia, who made the facilities of his crab house available for experimental work. In the Laboratory at Yorktown, Mrs. Mildred D. Sandoz and Mrs. R. Rogers-Talbert provided valuable assistance in the course of the work. Acknowledgment is also made to Professor George W. Oliver, Director of the Summer Session of the College of William and Mary in Virginia for helping to arrange for this study.

The Cranbrook Institute of Science, where the paper has been prepared for publication, has generously provided necessary facilities and aid for assembling the data. Thanks are also expressed to Professor P. S. Dwyer of the Statistical Laboratory of the University of Michigan for helpful advice.

METHODS

Width (W) measurements of large crabs were made with a specially constructed measuring board permitting a record accurate to within the nearest half millimeter. A vernier caliper was used for taking linear measurements of small crabs (Fig. 1). Thickness (T) is a

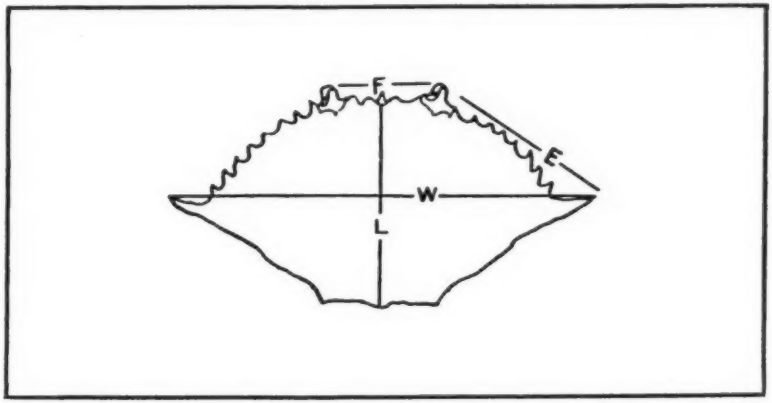

FIGURE 1
Outline drawing of the Blue Crab, *Callinectes sapidus* Rathbun, showing the dimensions measured. W = width; L = length; E = distance between the first and ninth antero-lateral spines of the right margin of the carapace (eye to spine distance); and F = distance between the preorbital spines (interocular width).

measure of the maximum distance between the dorsa surface of the carapace and the ventral surface of the cephalothorax when the crab is held in a horizontal position. Small crabs were weighed to the nearest milligram or fraction thereof and large specimens to the nearest decigram.

The crabs were landed at the dock by commercial boats and then steamed under a pressure of about 30 pounds per square inch for approximately 18 minutes in accordance with commercial practices. It was then possible to remove all the meat with a minimum of difficulty.

The data treated here include width, length (L), thickness and total weight (Wt) measurements of 99 male crabs, 60 ranging in width from 12 to 68 mm and 39 ranging from 135 to 201 mm; and 138 females, 79 having a width range of 12 to 58 mm and 59 with a

TABLE I

A series of mean widths of Blue Crabs, *Callinectes sapidus* Rathbun, having similar width dimensions, presented as a way of summarizing the individual measurements plotted logarithmically in Figures 1 and 2. Measurements expressed in *mm* and *gm*.

MALE						FEMALE					
Range of width	No.	Mean width	Mean length	Mean thickness	Mean weight	Range of width	No.	Mean width	Mean length	Mean thickness	Mean weight
12.0- 15.0	2	13.5	7.5	3.5	0.181	12.0- 15.0	5	14.3	7.7	4.0	0.266
17.2- 18.0	3	17.7	9.6	5.0	0.426	16.0- 19.5	9	17.5	9.5	4.9	0.447
19.0-	3	19.0	10.0	5.0	0.597	20.0- 22.0	7	21.0	11.2	5.7	0.785
20.0- 23.0	4	21.5	12.0	6.2	0.894	24.0- 27.5	5	25.7	13.5	7.1	1.443
24.0- 25.0	4	24.2	13.4	7.1	1.759	28.0- 30.0	8	29.1	14.9	7.9	2.071
26.0- 27.0	4	26.6	14.0	7.3	1.690	31.0- 33.5	7	32.0	16.2	9.1	2.865
28.0- 28.5	4	28.1	14.7	7.6	2.069	34.0- 37.2	7	35.4	18.3	9.9	3.869
29.0- 30.0	4	29.1	14.7	8.2	2.192	38.0- 41.6	14	39.2	19.5	10.9	5.122
31.0- 32.0	5	31.6	16.4	8.8	2.560	42.0- 44.0	6	43.0	21.6	11.9	6.444
33.0- 34.0	4	33.5	17.6	9.5	3.395	46.0- 49.5	5	47.5	23.6	13.0	8.513
35.0- 38.0	5	36.5	18.8	10.0	3.964	50.0- 52.5	4	50.9	25.2	14.6	10.533
40.0- 45.0	10	43.2	21.7	11.6	6.692	55.5- 58.0	2	56.7	28.5	16.0	13.664
46.0- 48.0	4	47.5	23.7	13.7	8.725	134.0-143.0	8	140.5	58.6	34.7	119.73
53.0- 56.0	3	54.3	31.0	15.7	14.824	145.0-147.0	5	146.0	60.9	36.4	133.81
68.0-	1	68.0	34.0	20.0	28.776	150.0-154.0	4	152.0	61.4	36.4	139.59
135.0-150.0	3	141.3	63.8	36.8	165.13	155.0-159.0	9	156.4	64.0	37.4	157.07
154.0-157.0	8	154.7	68.3	39.1	204.65	160.0-164.0	17	161.8	65.6	38.0	158.61
159.0-165.0	7	163.3	69.9	40.4	210.50	165.0-167.0	8	165.5	65.9	39.7	156.04
166.0-173.0	9	170.0	71.7	41.3	215.82	169.0-171.0	5	169.8	66.8	39.2	180.83
175.0-179.0	5	177.4	72.9	42.6	256.29	175.0-179.0	2	177.0	67.2	41.0	179.86
182.0-186.0	4	184.0	77.1	43.6	293.46	185.0-	1	185.0	70.0	41.0	225.62
190.0-191.0	2	190.5	77.5	43.5	190.08						
201.0-	1	201.0	90.0	44.0	271.27						

range of 134 to 185 mm (Table I). Lack of material for the middle range weakens the certitude of the growth curve (Figs. 2-3). How-

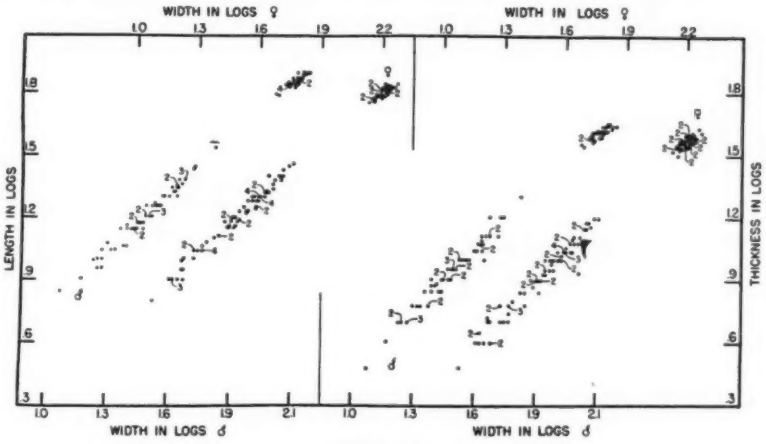

FIGURE 2
Showing the length-width and thickness-width relations of *Callinectes sapidus* Rathbun ranging in width from 12 to 201 mm for males and from 12 to 185 mm for females.

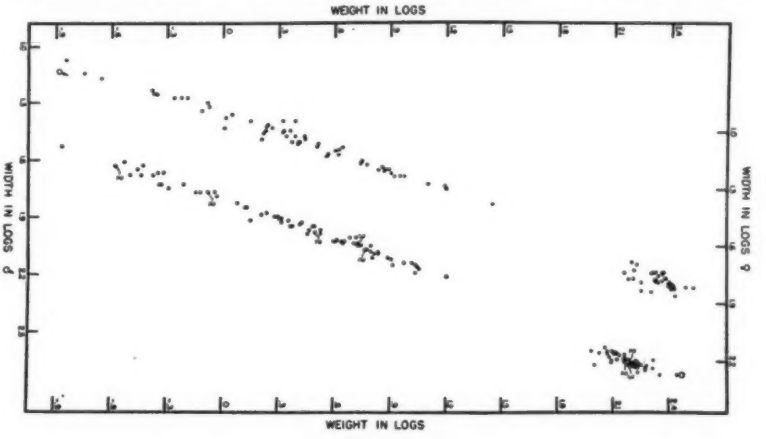

FIGURE 3
Showing the weight-width relations of *Callinectes sapidus* Rathbun ranging in width from 12 to 201 mm for males and from 12 to 185 mm for females.

ever, as is shown later (Table III), there is good reason for believing that the fitted curve is a reasonably accurate representation of the growth trend for this species. Growth, therefore, follows the law of allometry as expressed by the well-known formula of a constant differential growth ratio:

$$Y = aX^b$$

(Table II). The weight-width relationship has been described by

TABLE II

Values of constants a and b in equations of type $Y = aX^b$ expressing size relationships of width (W), length (L), thickness (T), and weight (Wt) in *Callinectes sapidus* Rathbun. For comparative purposes constants of differential growth ratios for grouped data on length and eye to spine (E) taken from Newcombe *et al.* (1949) are included.

Ratios	Sex	Range of width mm	n	a Values	b Values
L-W Ungrouped	males	12-201	99	0.772	0.885
	females	12-185	138	0.818	0.862
L-W Grouped	males	10-189.9	18	0.698	0.910
	females	10-189.9	18	0.747	0.888
T-W Ungrouped	males	12-201	99	0.343	0.934
	females	12-185	138	0.363	0.918
E-W Grouped	males	10-179.9	16	0.313	1.049
	females	10-179.9	17	0.322	1.045
Wt-W Ungrouped	males	12-201	99	263×10^{-6}	2.669
	females	12-185	138	343×10^{-6}	2.575

using the "ponderal index" of Thompson ('45, p. 194) obtained by dividing total weight by width cubed or by the product of width times length times thickness (Fig. 6).

Weights of the several parts of the body (but not total weights) are confined to a relatively narrow width range, namely 135-201 mm for males, 146-179 mm for females without a "sponge", and 134-185 mm for females with a "sponge" (Table V). The designation "remainder" indicates the visceral mass and skeletal parts which comprise a large percentage of the total weight. A total of 28 males, 20 females with "sponges" and 20 females without "sponges" were studied, one half of the total by each of the junior authors. These groups were selected from a relatively narrow width range so that the arithmetic mean widths (161.9 mm for males, 162.5 mm for females, and 158.3 mm for females with "sponge") would be as nearly as possible the same to permit a satisfactory comparison of males with the two

female groups (Table VII). Data for the 20 smallest male crabs were selected from the total of 28 and comparable arithmetic means determined. The mean widths are *not* significantly different, hence the two linear measurements and six independent weight measurements may be directly compared.

Results

The findings of this study confirm and supplement the observations by Newcombe *et al.* ('49) in respect to the constant differential growth ratio of the relative rates of growth in length and width. Knowledge of linear growth relations has been extended by measurements of thickness (T). Also, a beginning has been made in the study of weight characteristics of *Callinectes sapidus* considered in relation to corresponding linear dimensions. Perhaps the most comprehensive data are those comparing various linear and weight dimensions in each of three different types of crabs and revealing the magnitude of size differences between these types.

Linear measurements over a wide range of size. Length-width relations were studied by Newcombe *et al.* ('49) who used grouped data which, for certain analyses, are inferior to individual observations. By plotting several linear measurements in a variety of ways it was demonstrated that the growth of these dimensions is expressed by the allometric equation: $Y = aX^b$. Using a double logarithmic grid either a single straight line or two straight lines were required to fit the entire range of the data. The existence of two slightly different stanzas indicating a change in the differential growth ratios at a width of around 25 mm was found in the case of the length-width data. The rather extensive data consisted of arithmetic means of groups of individual measurements. The value of the growth coefficient, b, which is the ratio of the percentage growth rates of the dimensions studied, and that of the initial growth index, a, which in turn is the ratio

$$\frac{Y}{X^b}$$

are given in Table II.

The present ungrouped data deal also with length-width relations and, in addition, the thickness-width and weight-width relationships (Table II). Knowing that the growth dimensional ratios of this crab

undergo a significant change at a width of about 25 mm, it is possible to interpret the distribution of the points plotted logarithmically in Figure 2. It is assumed that the same explanation holds for the slight divergence from a straight line in the smaller size measurements plotted in Figure 3. The suitability of the allometric expression is also indicated by the similarity of the actual and calculated values given in Table III. The comparison of the growth coefficients ob-

TABLE III

Comparison of calculated with actual values for linear (mm.) and weight (gm.) dimensions, width being the independent variable. Calculated values are based on constants given in Table II. Actual values are those of Table VI.

	ARITHMETIC MEAN WIDTHS					
	Males 161.9 mm		Males 168.9 mm		Females 162.5 mm	
	Actual	Calculated	Actual	Calculated	Actual	Calculated
Length	69.3	69.6	71.9	72.3	65.2	65.9
Thickness	40.0	39.9	41.0	41.5	38.9	38.9
Weight	205.2	206.9	220.2	231.7	164.7	169.2

tained from the grouped and ungrouped data given in Table II shows that they are in good agreement. Values of the growth coefficient, b, for the length of males and females, namely 0.885 and 0.862 were tested for heterogony (allometry) with respect to body width. This was done by calculating the Student Fisher "t" (Rider, '39, p. 95) and results were considered to be significant if the P value was less than 0.01. Both values of b were found to be significantly different from 1 at the one percent level, the values of t being 16.4 and 29.6 respectively, thus establishing negative heterogony.

Interest lies in the growth dimensional ratios that show significant sex differences. Test of difference in the values of b for length in males and females have shown that they differ significantly at the one percent level ($t = 2.93$) (compare Olmsted and Baumberger, '23; and MacKay and Weymouth, '35).

Thickness-width growth relations were analyzed for allometry and found to conform to the common parabolic equation (Fig. 2). The values of b were tested for significance of their differences from unity. In males $t = 8.8$ and in females, $t = 12.4$ indicating that the respective b values of 0.934 and 0.918 are significantly different from one at the one percent level. No significant sex difference was found, the value of t being 1.5 indicating insignificance at the 5% level.

In both length-width and thickness-width regressions, tests were made for the significance of differences between the values of the "initial growth index", a, for males and females. These gave t values of 1.72 and 1.26 indicating no significant differences at the one percent level. Figure 4 shows the trend of changes in form accompanying growth. The eye to spine dimension[2] exhibits positive allometry ($b = 1.05$) whereas length and thickness are negatively allometric, the constant growth coefficients being 0.88 and 0.93.

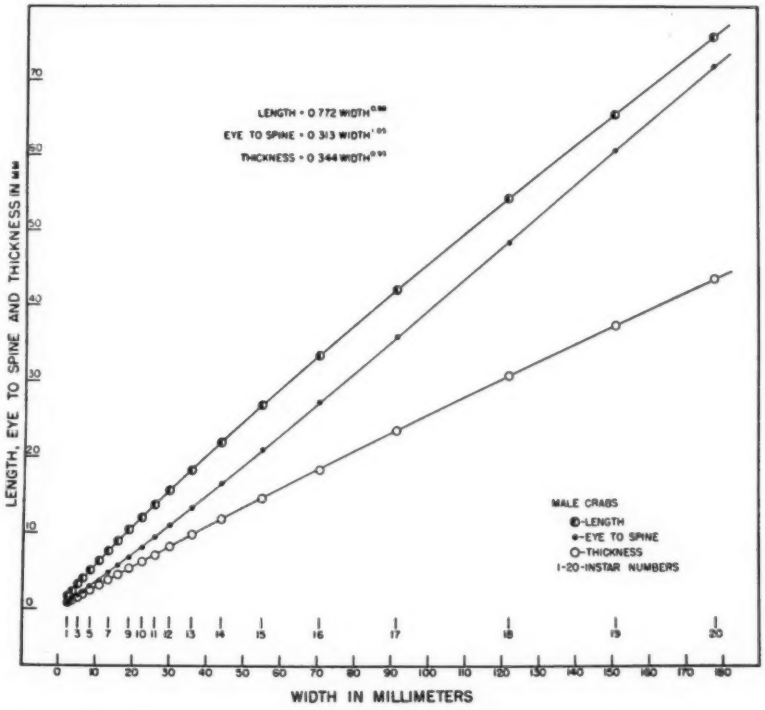

FIGURE 4

Showing changing form in *Callinectes sapidus* Rathbun with increase in size at the successive instars. Curves based on length-thickness data given in Figures 2 and 3, and in Tables I and II.

[2]Reference is made to the distance between the first and ninth antero-lateral teeth of the margin of the carapace. This dimension, treated elsewhere (Newcombe *et al.*, '49), is included here and in Table IV for comparative purposes.

Total weight measurements over a wide range of size. The rate of increase in weight with growth in width is of especial interest, theoretically and practically (Table I, Fig. 5). A growth of 12% in width in a crab 170 mm wide is accompanied by a gain in weight of 35%. As anticipated the weight-width relation was found to be expressed by the parabola $Wt = aW^b$, the growth coefficients, b, for males and females being 2.669 and 2.575, respectively (Table IV and Fig. 5). These exponents are both significantly different from three at the one percent level, the value for t for males being 14.1. We have also tested for significance of the sex differences in values of b and obtained for t a value of 3.3 indicating a significant difference at the one per cent level. However, differences in the value of a ($t = 2.2$) are not significant at the one percent level.

At a width of 190 mm males are about 25% heavier than females without "sponges" whereas at a width of 90 mm the corresponding percentage is 17. The correlation coefficients for logarithms of the length-width, thickness-width, and weight-width data for male crabs were found to be 0.997, 0.997, and 0.996, respectively. At this point it should be indicated that the weight regression for females is based on a combination of weights of females without "sponges" and weights of females with "sponges" minus the weight of the "sponge". Whether or not weight of the "sponge" is included, the data will be unsatisfactory for comparative purposes. This subject is discussed on pages 92 and 93.

Weights of the several instars. Gray and Newcombe ('38b) and Newcombe *et al.* ('49), on a basis of over 800 observations of moulting crabs, estimated the average width and number of the instars for the Blue Crab. The widths of the 20 instars for males (only), given in Table IV, were obtained directly from a graph of the original data. Corresponding linear and weight dimensions for males and females (without "sponges") have been calculated by means of constants given in Table II. The range of widths on which the weight-width calculations are based is 12 to 201 mm for males, and 12 to 185 mm for females. Therefore, the weights for the instars below 12 mm in width are purely estimations. The principal differences in weight between the sexes are in the larger crabs. Calculated values for the length, eye to spine and thickness dimensions of the instars are likewise given in Table IV. Growth trends of male crabs are

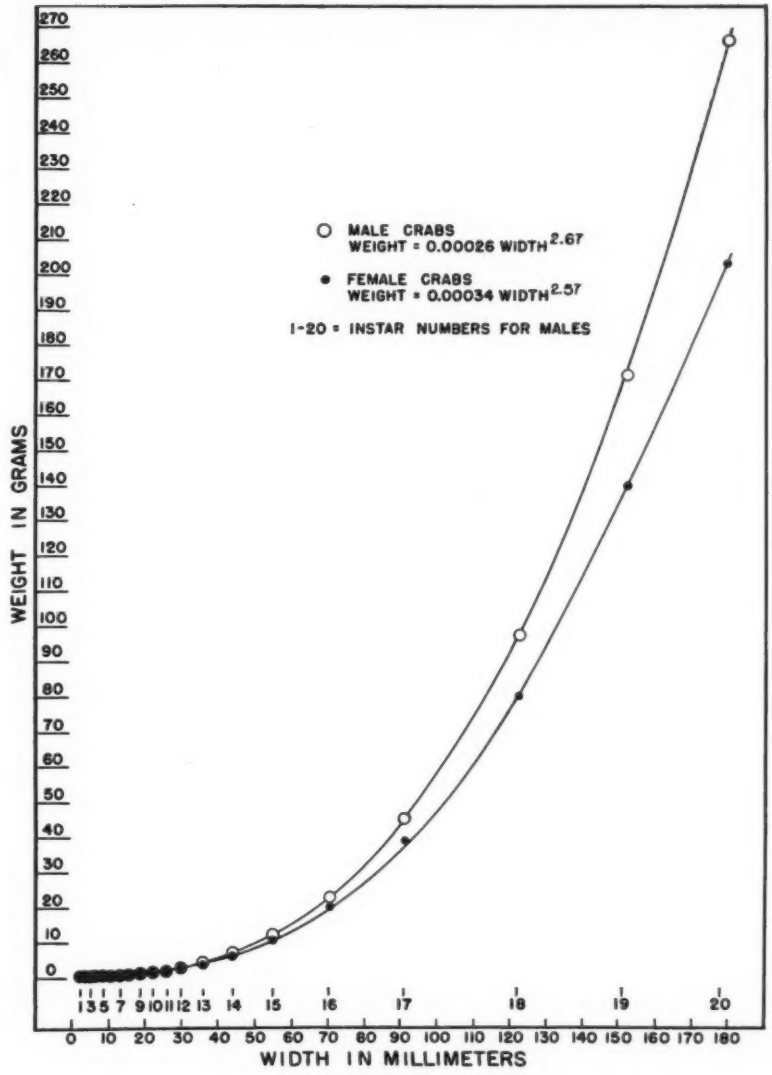

FIGURE 5

Showing trends of growth in weight that accompany increase in width of male and female Blue Crabs, *Callinectes sapidus* Rathbun. Widths of instars of male crabs *only* are indicated by numbers 1 to 20. Curves based upon data given in Figures 2 and 3, and in Tables I and II.

TABLE IV

Linear and weight dimensions of the different instars of *Callinectes sapidus* Rathbun calculated from the widths obtained from a curve of best fit based on original data for males.

		UNGROUPED						GROUPED			
		a=0.772 b=0.885	a=0.818 b=0.862	a=0.344 b=0.934	a=0.363 b=0.918	a=0.00026 b=2.669	a=0.00034 b=2.575	a=0.747 b=0.888	a=0.698 b=0.910	a=0.322 b=1.045	a=0.313 b=1.049
Instar No.	Width of Instars	♂ Length	♀ Length	♂ Thickn.	♀ Thickn.	♂ Weight	♀ Weight	♀ Length	♂ Length	♀ Eye to spine	♂ Eye to spine
1	2.5	1.74	1.47	0.81	0.80	0.0030	0.0036	1.68	1.61	0.84	0.82
2	3.7	2.46	2.53	1.17	1.21	0.0086	0.0097	2.39	2.29	1.26	1.24
3	5.1	3.26	3.33	1.58	1.62	0.0203	0.0228	3.17	3.07	1.77	1.73
4	6.6	4.10	4.26	2.01	2.05	0.0404	0.0442	3.99	3.89	2.31	2.27
5	8.6	5.18	5.23	2.57	2.62	0.0820	0.0875	5.05	4.94	3.05	2.99
6	11.0	6.44	6.46	3.23	3.28	0.1581	0.1649	6.28	6.19	3.94	3.88
7	13.5	7.72	7.71	3.92	3.96	0.2730	0.2793	7.52	7.45	4.88	4.80
8	16.0	8.98	8.93	4.59	4.63	0.4296	0.4326	8.76	8.70	5.83	5.74
9	19.0	10.46	10.35	5.39	5.42	0.6800	0.6766	10.21	10.17	6.98	6.88
10	22.5	12.14	11.98	6.31	6.33	1.068	1.065	11.85	11.87	8.33	8.21
11	26.0	13.80	13.57	7.22	7.23	1.570	1.509	13.48	13.53	9.69	9.55
12	30.0	15.66	15.35	8.26	8.25	2.300	2.183	15.31	15.41	11.26	11.10
13	36.0	18.41	18.38	9.79	9.75	3.743	3.492	17.99	18.20	13.61	13.45
14	44.0	21.99	21.36	11.81	11.73	6.396	5.855	21.50	21.84	16.79	16.59
15	55.0	26.79	25.89	14.54	14.39	11.60	10.40	26.22	26.76	21.19	20.97
16	70.5	33.36	32.06	18.34	18.07	22.50	19.71	32.68	33.55	27.48	27.20
17	91.5	42.02	40.14	23.40	22.96	45.12	38.56	41.19	42.52	36.08	35.76
18	122.0	54.21	51.45	30.61	29.90	97.27	80.91	53.19	55.25	48.74	48.37
19	151.0	65.46	61.83	37.36	36.36	171.90	140.1	64.28	67.08	60.91	60.49
20	178.0	75.71	71.24	43.56	42.39	266.50	214.0	74.39	77.91	72.33	71.88

shown in Figs. 4 and 5. Moulting estimates are, of course, based on averages and there is a great deal of variation. This variation is increased with increase in age since a small difference in width in young crabs will be magnified until in the adults the difference is highly conspicuous.

Body weight in relation to linear dimensions. The above data have been treated so as to provide a reasonable basis for predicting from a particular width measurements other linear dimensions and also the corresponding total body weight. A comparison of the weight-width relations in crabs of different sizes is also given by the weight-width coefficient sometimes called a "coefficient of condition" or "form index" or "crude index of body build" or the "ponderal index" in which $Wt = kL^3$, k being the weight-width coefficient (Thompson, '43). We have used the expression

$$k = \frac{Wt}{W^3} \times 10^7$$

and also

$$k_1 = \frac{Wt}{W \times L \times T} \times 10^6$$

Fig. 6). It is seen that the values of k and k_1 decrease gradually with increase in size. The calculated value of k_1 for the mean width 161.9 mm for males (Table V) is 457 while the corresponding value obtained from the curve in Figure 6 is about 461, a close approximation. In females without "sponges" of average width of 162.5 mm, the corresponding values of k_1 are 400 and 402, respectively. The original weight-width curve for females, on which data the curves in Figure 6 are based, has been constructed on weight data for females made up of weight of female crabs without "sponges" and the weight of females with "sponges" minus the "sponge" weight, these two groups being present in about equal numbers. The value of k_1 for females of width = 158.3 mm read from the curve in Figure 6 is about 410 and the corresponding value calculated from means given in Table V is 391. Using as a total weight the body weight plus the "sponge" weight, namely 177.5 gm instead of 146.3 gm we get a value for k_1

[3]Mrs. Sue B. Christian worked with one of the writers (C.L.N.) at Yorktown during the summer of 1943 and obtained some valuable preliminary weight data for the Blue Crab.

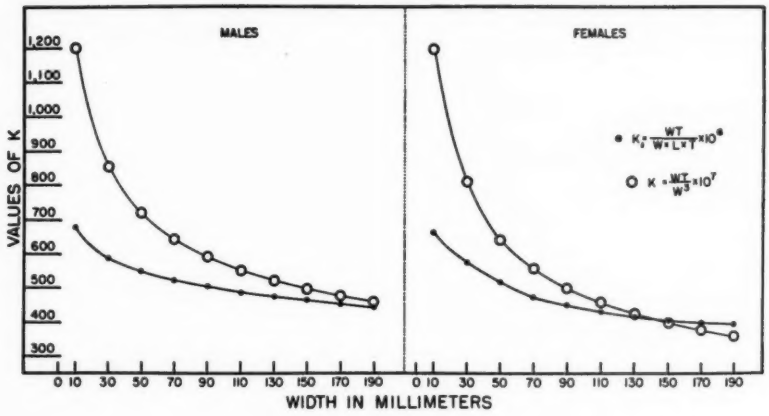

FIGURE 6
Showing trends in the magnitude of two types of "form index" applied to male and female Blue Crabs, *Callinectes sapidus* Rathbun, over a wide range of size.

of 475 indicating that the "sponge" weight is, in large part, supplemental weight and not entirely a compensatory one. It is pointed out later (see pp. 91, 92) that much of the "sponge" weight is probably due to interstitial water and that of the average weight per "sponge" of 31.2 gm, about 16.1 gm represents the weight of ova extruded when the "sponge" was formed. Thus, by adding this weight to the weight of the body minus the weight of the "sponge", we get 146.3 + 16.1 or 162.4 gm. The value of k_1 corresponding to a weight of 162.4 gm is 434 which is about half way between 393 and 475 and seemingly a fairly representative value of k_1. The value of k_1, namely 410, estimated from the curve in Figure 6 is expectedly low due to the influence of the crab weights obtained by deducting the "sponge" weights.

Weight of body parts in males and females with and without "sponges". Comparative data for three linear and six weight (including the "sponge") measurements, all of which are independent, are given in Table V. The duplicate groups provide good checks and reflect a high degree of dependability of the technics employed. Adult male crabs are relatively heavier in all respects studied than females. In males, the total meat weight is approximately 38% of the total body weight whereas in females it drops to 32% and 28%

TABLE V

Comparison of arithmetic means of measurements of size characteristics of two comparable groups of males, females with "sponges" and females without "sponges" of *Callinectes sapidus* Rathbun

	n=10 Females with sponge		n=10 Females without sponge		n=14 Males		n=20 Females with sponge	n=20 Females without sponge	n=20 Males narrowest 20 of	n=28 Males
	Group I	Group II	Group III	Group IV	Group V	Group VI	Groups I & II	Groups III & IV	Groups V & VI	Groups V & VI
Width	157.9	158.7	162.4	162.7	170.1	168.1	158.3	162.5	161.9	168.9
Length	63.5	63.7	65.2	65.3	73.1	70.9	63.6	65.2	69.3	71.9
Thickness	37.5	36.9	38.9	39.0	40.8	41.3	37.1	38.9	40.0	41.0
Total weight*	142.0	150.7	166.5	162.8	219.0	221.6	146.3	164.7	205.2	220.2
Total of lump meat	13.2	13.0	12.0	13.0	18.7	18.4	13.1	12.4	17.5	18.6
Total of flake meat	19.7	20.2	20.8	21.1	28.9	28.9	19.9	20.9	27.5	28.9
Total of claw meat	12.4	13.5	13.9	14.3	26.8	24.8	13.0	14.2	24.9	25.8
Total comm. meat wt.	45.3	46.7	46.7	48.4	74.4	72.1	46.0	47.6	70.0	73.3
Other meat weight	3.4	4.5	4.3	5.0	8.5	9.4	3.9	4.6	8.7	8.9
Total meat weight	48.7	51.2	51.0	53.4	82.9	81.5	50.0	52.3	78.7	82.2
Sponge weight	30.3	31.9					31.2			
Remainder*	93.3	99.5	115.5	109.4	136.1	140.0	96.3	112.4	126.6	138.1

*Not including the sponge.

TABLE VI

Showing several linear and weight dimensions of three types of Blue Crabs expressed in percentages. Each of the 11 means given for the three types represents the average of the means of two groups, each containing 10 crabs.

Dimensions	Males		Females with "sponges"		Females without "sponges"	
	Means	Percentages	Means	Percentages	Means	Percentages
Width	161.9		158.3		162.5	
Length	69.3	42.8	63.6	40.1	65.2	40.1
Thickness	40.0	24.7	37.1	23.4	38.9	23.9
Total weight	205.2		177.5		164.7	
Weight of Lump Meat	17.5	8.5	13.1	7.4	12.4	7.5
Weight of Flake Meat	27.5	13.4	19.9	11.2	20.9	12.7
Weight of Claw Meat	24.9	12.1	13.0	7.3	14.2	8.6
Total Weight Commercial Meat	70.0	34.1	46.0	25.9	47.6	28.9
Total Weight Other Meat	8.7	4.2	3.9	2.2	4.6	2.8
Total Weight of Meat	78.7	38.3	50.0	28.1	52.3	31.7
Weight of Remainder of Crab	126.6	61.7	96.3	54.3	112.4	68.2
Weight of "Sponge" Mass			31.2	17.6		

depending on the absence or presence of a "sponge" (Table VI). In the range covered, length varies from about 40 to 43% of the width and thickness is from approximately 23 to 25%. The remainder of the crab which is largely shell comprises about 62% of the total weight in males and 68% in females without "sponges" and 66% in those bearing a "sponge" if the "sponge" weight is excluded.

Table VII shows the character of the data on which conclusions may be formulated. The data provide excellent comparisons for signifying sexual similarities and dissimilarities, and for contrasting various linear and weight characteristics of adult crabs of a particular sex. The fiducial limits based on a two percent probability are given. From a statistical viewpoint it is of interest to compare the 10 variables of the three groups of similar widths to determine the significance of observed differences. Table VIII shows that males and females are significantly different at the one percent level in respect to all the variables with the exception of thickness and remainder weight. Females with and without "sponges" are significantly differ-

TABLE VII

Showing the representative character of the mean values used in comparing the size characteristics of male and female specimens of *Callinectes sapidus* Rathbun with and without "sponge". n = 20. Size is expressed in millimeters.

					MEAT WEIGHTS						
	Width	Length	Thick-ness	Total Weight	Lump	Flake	Claw	Com-mercial	Other	Total	Weight of Remainder
MALES	135-	61-	35-	144-	12-	19-	13-	49-	5-	58-	78-
Size range	178	78	44	260	23	38	35	91	13	100	160
Mean size	161.9	69.3	40.0	205.2	17.5	27.5	24.9	70.0	8.7	78.7	126.5
Standard error	2.67	0.87	0.53	7.76	0.80	1.29	1.27	2.83	0.50	3.05	5.36
U.E. of S. Deviation*	11.93	3.89	2.36	34.70	3.60	5.79	5.66	12.64	2.23	13.66	23.96
Fiducial Limits	155.1-	67.1-	38.7-	185.5-	15.5-	24.2-	21.7-	72.8-	7.4-	70.9-	113.0-
	168.7	71.5	41.3	224.9	19.5	30.8	28.1	77.2	10.0	86.4	140.2
FEMALES	146-	60-	36-	133-	10-	15-	9-	36-	3-	40-	93-
Size range	179	70	43	209	15	25	19	58	7	64	148
Mean size	162.5	65.2	38.9	164.7	12.4	20.9	14.2	47.6	4.6	52.3	112.4
Standard error	2.05	0.63	0.38	4.42	0.33	0.65	0.64	1.28	0.24	1.47	3.21
U.E. of S. Deviation*	9.16	2.82	1.68	19.76	1.47	2.91	2.86	5.71	1.09	6.58	14.37
Fiducial Limits	157.3-	63.6-	37.9-	153.5-	11.6-	19.2-	12.6-	44.3-	4.0-	48.6-	104.3-
	167.7	66.8	39.9	175.9	13.2	22.6	15.8	50.9	5.2	56.0	120.5
FEMALES WITH "SPONGES"	134-	56-	33-	105-	10-	13-	8-	31-	1-	32-	73-
Size range	185	70	41	224	17	27	22	65	7	72	152
Mean size	158.3	63.6	37.1	146.3	13.1	19.9	13.0	46.0	3.9	50.0	96.3
Standard error	2.54	0.77	0.47	5.73	0.43	0.82	0.76	1.80	0.27	2.02	4.46
U.E. of S. Deviation*	11.36	3.44	2.10	25.64	1.94	3.66	3.42	8.04	1.19	9.03	19.96
Fiducial Limits	151.8-	61.6-	35.9-	131.7-	12.0-	17.8-	11.1-	41.4-	3.2-	44.9-	85.0-
	164.8	65.6	38.3	160.8	14.2	22.0	14.9	50.6	4.6	55.1	107.6

*Unbiased estimate of standard deviation.

TABLE VIII

Student Fisher values of t indicating significant differences between sexes. 0 star = significant difference at the 1 percent level; 1 star = no significant difference; 2 stars = significant difference between the 2 and 5 percent levels; and 3 stars = significance between the 1 and 2 percent levels. Comparisons based on groups having similar mean widths.

Dimension	Males and Females	Males and Females with "sponges"	Females and Females with "sponges"
Width	0.18*	0.99*	1.30*
Length	3.82	4.96	1.66*
Thickness	1.70*	4.10	2.99
Total Weight	4.54	6.10	2.54***
Lump Meat Weight	5.86	4.86	1.19*
Flake Meat Weight	4.59	4.96	0.91*
Claw Meat Weight	7.54	8.04	1.82*
Total Commercial Meat	7.22	7.15	0.70*
Other Meat	7.31	8.41	1.94*
Total Meat	7.79	7.84	0.92*
Weight of Remainder	2.27**	4.60	3.22

ent at the one percent level only in respect to thickness and remainder weight.

Discussion

Constancy of form in growing crabs may be demonstrated within certain size limits or within particular physiological periods. It is an interesting fact that constant differential growth ratios occur but more so that they change in accordance with particular conditions. This much has been demonstrated by various investigators with different degrees of emphasis. Linear as well as weight ratios are known to change with the onset of sexual maturity. For example, in *Callinectes sapidus,* the width of the abdomen in females increases greatly in proportion to body width during the final moult but this relation has not been studied quantitatively. It seems to represent a sudden modification that is invariably associated with sexual maturity (compare Tazelaar, '30). The size at which sexual maturity is reached varies and hence any plotting of data pertaining to a certain population will represent the change in the form of a curvilinear regression although the transformation itself is not a gradual phenomenon. Variations in width of the abdomen in immature Fiddler Crabs was exhaustively studied by Morgan ('23), while Sandon ('37) and others have shown the magnitude of differential growth in particular appendages of crabs and other crustacea.

Relatively few data are available on the weight of the body muscles in relation to the whole body at different moults or even different sizes. Here, a beginning has been made by treating total weight in males and females throughout a wide range of size, and a variety of meat weights in adults, covering a relatively narrow range of width.

Regarding linear growth dimensional ratios, males are shown to be longer but not thicker, relative to their width, than females. Newcombe *et al.* ('49) reached this conclusion for the length dimension using grouped data. Employing ungrouped data we have obtained constants for the allometric equation similar to those based on grouped data, namely $b = 0.885$ and 0.862 for males and females, respectively (Table II). In the length-width and thickness-width regressions there is no significant difference at the one percent level between males and females in respect to the "initial growth index", a. Negative heterogony for males and females as far as the length-width relation is concerned has been established (Fig. 4). Olmsted and Baumberger ('23) found the length-width curve to be a straight line in three species of grapsoid crabs. However, Huxley ('32, p. 261) questioned the absence of heterogony (allometry) in their material in the belief that their observations were not sufficiently numerous to establish the point in question.

While the data on total weights (Table I) are limited in their spread within the central part of the range, they are regarded to be fairly representative as shown by various direct and indirect checks (Table III). It has been demonstrated that the law of allometric growth holds for several linear dimensions and that in some instances two or more stanzas are recognizable (Newcombe *et al.*, '49). A width of about 25 mm was found to be a size at which the changes in differential growth rates take place. This is suggested by the position of the lower points of the size range shown in Figures 2 and 3, and doubtless would be more conspicuous were measurements of smaller specimens plotted. Over most of the width range studied it is seen that the points in the figures closely approximate straight lines. Thus, the allometry relation is upheld and we have a convenient tool for dimensional analyses in this species as well as for prediction purposes. The fact that many of the weights of adult females which were used in our calculations were those of "sponge" crabs, less the weights of the "sponges", probably has given a b value, namely 2.575, somewhat

lower than would have resulted from using only those females that lacked the "sponge" (see pp. 91, 92). That the effect is not great is indicated by the fact that the calculated values are already somewhat in excess of expectation as indicated in Table III. The *b* values for the weight-width relation in males and females are significantly less than three and significantly different from each other.

The method for estimating the number of moults and the size of each instar has been referred to. The average amount of increase in width at each moult for males and females has been found to be 26%. In other words, the width increases by the $\sqrt[3]{2}$ or by the ration 1 : 1.26. This corresponds exactly with Przibram's law and is approximately what Brooks found for *Squilla* (Brooks, 1886 and Przibram, '31). However, the average weight ratio of increase is not 2 but 1.81. Without change in form, a body that is doubled in volume will have increased in width by the cube root of two. In the instance of the crab, width increases by the $\sqrt[3]{2}$ but the weight is not doubled.

It should be pointed out that primary impetus to the application and use of the allometric equation was provided by Huxley ('32), and, significantly, that Keys ('28) tried "the general type equation $Y = a(X)^n$" in his study of Fundulus, herring and sardines (compare Brody, '45, p. 607). Keys made a valuable analysis of the utility of the equation and pointed out that the cube law accepted by Hecht ('16) while of practical value, is an incorrect formulation of the weight-length relation which is more accurately expressed by the allometric equation that has very different theoretical implications (compare Hile, '36). Recent fish studies have shown that weight usually increases more rapidly than the cube of the length. Since the specific gravity of a fish is not likely to change, the form probably changes as the organism grows. Keys established a positive correlation between *a* and weight indicating that weight increases more rapidly than the cube of the length. In the crab the growth coefficient, *b*, for the weight-width relation, is significantly less than three in both sexes. This indicates a difference in specific gravity or a difference in form or both. We do not possess specific gravity data but such records were made by Olmsted and Baumberger ('23) who failed to find a difference in specific gravity between *Hemigrapsus nudus*, *H. oregonensis* and *Pachygrapsus crassipes*. This might indicate an

unlikelihood of significant variation in a single species, but the possibility of some change in the specific gravity of a crab during its lifetime cannot be overlooked. They live in high salinity water, in all degrees of estuarine conditions, and may spend long periods in fresh water. In any case changing form in *Callinectes sapidus* has been demonstrated and we believe it to be at least partly responsible for the low values of the growth coefficient. Separation of such variables as specific gravity, body form, growth coefficients of the linear dimensions, and differential weights of body parts as they apply to related and unrelated animals, is an extremely complex problem. The index of condition has been extensively used for descriptive purposes in fish (Hile, '36) and we have applied it to Blue Crabs over a wide range of size. The effect of maturity on the index of condition in females is minimized in the curves of Figure 6. This effect is shown by contrasting the values of k_1 for females without "sponge" and for females with "sponge". The former group having a mean width of 162.5 mm is largely made up of gravid females and the value of k_1 is 400. The latter group, with a similar mean width, namely 158.3 mm, has a k_1 value of 475 with the "sponge" and 391 without the "sponge" (Table V).

Fig. 6 shows that weight decreases in relation to W^3 much faster than in relation to $W \times L \times T$, the ranges of k and k_1 in males being 1200-463 and 678-450, respectively. In the case of both males and females the values of k and k_1 tend to approach each other with increase in size. However, in females the increase in weight in relation to increase in both W^3 and $W \times L \times T$ is less than in males. This bears out the relation shown in Figure 5.

Hile ('36) gives a thorough analysis of the use of the two equations W (weight) $= kL^3$ and $W = CL^n$, the types used in this paper. He points out that "coefficients of condition calculated from the cube relationship describe relative heaviness independently of the general length-weight relationship, and are more satisfactory measures of condition than the quantity C [our a] in the equation $W = CL^n$ where the value of n [our b] is determined empirically."

Weight of body parts in adults. Apart from estimates of the weight of commercial meats in particular species, there seems to be little, if any, accurate data on the weight of the internal parts of any crustacean. In this respect, the present study falls far short of com-

pleteness. However, the measurements of adults of both sexes provide interesting data. Males, females and females with "sponges" having similar widths possess certain unexpected points of difference. Males and females are, with the exception of thickness, different in respect to all the measurements considered (Table VIII). The remainder weights of the two sexes, composed mainly of skeletal parts, are not significantly different at the one percent level but are different between the two and five percent levels. The explanation lies in the fact, clearly shown by Mrs. Sue B. Christian,[3] that while males have much heavier shell weight than gravid females, the weight of the viscera parts, particularly the reproductive organs, almost compensates for it. Her data have shown that, in males of this width, the shell weight is approximately 70% of the remainder weight and in gravid females, about 52%. Males are larger in most respects whereas females with "sponges" are the smallest of the three groups. The chief point of difference between the two female groups lies in the difference in remainder weight (Table V). Total meat weight differs by only 2.3 gm while the remainder weight in females with "sponge" is 16.1 gm less (112.4 — 96.3 gm) than in those without a "sponge". Adding this weight to the total weight without the "sponge", namely 146.3 gm, gives 162.4 gm. This weight is a close approximation to the total weight (164.7 gm) of the other female group and similarity of the remaining weights would warrant this expectation. The implication, then, is that the remainder weight decreased to the extent of about 16 gm when the "sponge" was formed but the "sponge" in its external condition increased in weight to 31.2 gm due in large part to interstitial water. The supposition that formation of the "sponge" results in a reduction of the remainder weight to the extent of about 16 gm is supported by Mrs. Christian's results indicating that the weight of the visceral mass in "sponge" Blue Crabs ($n = 3$) is about 15 gm less than that in gravid females ($n = 5$). This evidence indicates that the combination of female weight data referred to above may be expected to influence the value of b in the weight-width regression for females.

From a viewpoint of food production it is highly significant that there is no significant difference in the amount of commercial meat produced by females with and without "sponges". It appears that the production of the "sponge" is not accomplished at the expense

of the weight of commercial meat as several persons have postulated. The amounts of commercial meat available[4] for use in females with and without "sponges" are 31.4% and 28.9%, respectively, of the total weight of the crab (not including the "sponge").

In respect to all weight measurements studied male crabs are by far the heaviest per unit of width. The mean weight for males was found to be 205.2 gm as compared with 164.7 gm for females of the same width.

Summary and Conclusions

The analysis of linear and weight data for male and female Blue Crabs, *Callinectes sapidus* Rathbun, includes information on the nature of variation in size and form throughout life, the size of the instars believed to be characteristic of the species, the prediction equations for estimating several linear and weight dimensions from width, and lastly, the relations in adult crabs between three selected linear dimensions and six independent weight dimensions.

Evidence has been presented in support of the following conclusions:

1. Allometry is a fundamental characteristic of linear growth in *Callinectes sapidus* Rathbun. The length-width and thickness-width relations are expressed by the following equations: $L = 0.772W^{0.885}$ and $T = 0.343W^{0.934}$ for males; and $L = 0.818W^{0.862}$ and $T = 0.363W^{0.918}$ for females (Table II).

2. The length-width differential growth ratios in males differ significantly from those in females. No significant sex differences were observed in regard to the thickness-width ratios. Also, no significant sex differences at the one percent level was found in the initial growth index, *a*, for both the length-width and thickness-width regressions.

3. Changing body form represented graphically is shown by negative allometry for length and thickness and positive allometry for the eye to spine dimension in relation to width.

4. The growth coefficients of the parabola expressing the weight-width relations in males and females are 2.669 and 2.575, respectively. These coefficients are significantly less than three at the one percent level and they are also significantly different from each other.

[4]This amount is quite different from the amount of commercial meat actually removed by the commercial picker which is only about 65-70% of the total.

The average weight of male crabs, 170 mm in width, is 235.7 gm whereas females of this size weigh about 190.0 gm.

5. While the average increase in width accompanying moulting is in the ratio of 1 to 1.26, the $\sqrt[3]{2}$, the corresponding weight ratio is 1 : 1.81 rather than 1 : 2.

6. Male crabs in the 20th instar average about 266 gm in weight whereas females of this size are estimated to weigh approximately 214 gm.

7. Values for the coefficient of condition, as used here, vary from 678 in 10 mm crabs to 450 in 190 mm males using $W \times L \times T$. Corresponding values of 1200 and 463 were obtained by using W^3 in the equation (Fig. 6).

8. The effect of maturity on the index of condition, k_1 in females is shown by the fact that in crabs of comparable size females without a "sponge" had a k_1 value of 400, those with a "sponge" a k_1 value of 475. In the case of males of comparable size, the value of k_1 is 457.

9. During growth weight decreases in relation to the cube of the width much faster than in relation to the product of width, length and thickness.

10. Total weights of adult crabs averaging about 161 mm in width are as follows: males, 205 gm; females, 165 gm; and females with "sponge", 177 gm of which about 31 gm is the weight of the egg mass (Table VII).

11. In male crabs measuring about 162 mm in width, parts other than the muscle meats, i.e., visceral and skeletal parts, comprise about 62% of the total weight. The length and thickness dimensions are 43% and 25%, respectively, of the width.

12. The amount of meat in male crabs is around 50% greater than that in females of the same width. However, there is no significant difference in meat weight between females with and without "sponges". The difference observed in total weights, namely 177 gm and 165 gm, is considered to be due to absorption of water by the external egg mass and possibly to a difference in the quantity of eggs present (Table VI).

13. With the exception of thickness and remainder weight (visceral plus skeletal parts), male and female crabs are significantly different at the one percent level with respect to all the dimensions

measured (Table VIII). It has been indicated that the weights of the skeletal parts are significantly different.

14. Females with and without "sponges" are significantly different at the one percent level only in respect to thickness and remainder weight. Their total weights are significantly different at between the one and two percent levels. These weight differences reflect differences in the respective weights of the reproductive organs, the skeleton weights being quite similar.

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DEFINITION AND QUANTITATIVE EXPRESSION OF AGEING

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In the course of the history of biology, a great many theoretical views intending to interpret the concepts of growth, ageing, and senescence have been expressed. The oldest image proposed was based mostly on external observation, and divided life into three periods; first, a phase of youth in which anabolism is more important than catabolism, then a period of equilibrium in which anabolism matches catabolism, and finally, a period of senescence marked by the prevalence of catabolism, this phase eventually ending in death.

Rubner (1) proposed a "colloidal" theory of ageing, based on the simple physico-chemical model provided by inorganic chemistry: after a period of increase of the colloidal matter of the body (growth), ageing is characterized by the progressive dehydration and aggregation of the colloids. Minot (2), anticipating the formulation of the concept of "dynamic equilibrium" of the living matter, wrote that ageing is essentially characterized by a decreased percentage of growth rate so that "retardation of growth is old age and its cessation is death."

Brody, in his recent but already classical monograph (3), does take into account, when describing the ageing processes, such various factors as decreased enzymatic activity, decreased metabolic rate, decreased productivity of certain processes (e.g., milk production in the cow), and discusses the influence of certain factors on the rate of ageing such as temperature, over-feeding and partial starvation, etc. However, his paragraph on "criteria of ageing and the prime of life" does not reflect any clear pattern, but simply enumerates salient features of age; for instance, he mentions that "skin, hair, eyes, voice, the very movements of the body and of its parts, bear witness of the passing of years to chronological age." The assertion that "ageing may be represented quantitatively by the same exponential or mass

action or compound interest equation, $Y_1 = Ae^{kt}$, used for representing early growth", although it may be applied to certain processes (e.g., egg production), lacks generality because of the imprecise characterization of ageing.

The time has perhaps come to revise and define our thinking in connection with the concepts of growth and ageing in the light of recent experiments and new concepts of metabolism.

First, let us recall that growth is rightly defined as the enlargement of an organism by multiplication of reproducing units or increase in size of the cells. It is not generally synonymous with weight increase. Nevertheless, growth and weight increase are too often taken as equivalent, in feeding experiments for instance. This assumption may be justified in the case of young animals where increase in weight is not accompanied by important modifications in the composition of the organism. It is not justifiable in an older animal.

In a recent publication (4), this writer called attention to the fact that in a very young animal, an increase by 5 grams represents approximately 1 gram of protein and 4 grams of water and minerals, or an energy content of roughly 5 calories, while an increase by 5 grams in an older animal may represent 5 grams of fat, or 45 to 50 calories. Thus, one can easily see that from a thermo-chemical standpoint, a given weight increase may have widely different meanings depending on the age of the subject. The classical "growth" equation, $\frac{dw}{dt} = Kw$, (I), would seem to apply better to the energy content of the organism than to "growth", properly speaking.

A second pertinent consideration is that of the efficiency of food utilization for productive processes. If this ratio is expressed in grams of weight gained per gram of feed consumed, it can be shown to decrease rapidly with age. If, however, it is expressed in terms of energy retained by the organism to the energy of the food consumed, this decrease is considerably minimized, because the energy equivalent of the weight increase is progressively higher, attaining a value as much as 10 times that corresponding to very young tissues.[1]

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[1]This writer has shown, for instance (5, 6), that in the normal male rat of the "Yale" strain, the efficiency of food conversion to body weight in early growth decreased with time according to the equation:

$$E_t = 0.81 - 0.346 \log t \qquad \text{(II)}$$

where t is the time measured from weaning in days, E_t the efficiency in grams of

From these facts, it may be deduced that the characteristic biochemical phenomenon in the progressive cessation of growth and the onset of ageing process is not a sudden decrease in the efficiency of food utilization, but a passage from a type of food utilization directed toward protein synthesis to a type of utilization where fat synthesis is the dominant feature. This change of metabolic direction is represented on the classical "growth" curves by an inflection. From a physiological point of view, it corresponds to puberty, a period characterized by a profound change in the hormonal equilibrium of the body, and a shift in emphasis from the preponderance of the growth hormone, a protein hormone found in the water phase, to that of the lipoidal steroid hormone. What then would the expression of ageing be in the frame of this theory? If W is the total energy content of the organism at the time t, W_f the part of the energy content due to fat, then the instantaneous rate of ageing would be:

$$a_t = \left(\frac{\partial W_f}{\partial W}\right)_t \qquad \text{(III)}$$

or on a daily basis:

$$a_t = \frac{\Delta W_f}{\Delta W} \qquad \text{(IIIa)}$$

That this passage from protein synthesis to fat synthesis and this continuous increase of energy content can exist even though there may be no concommitant change in weight are confirmed by recent data obtained on man (7), on the rat (8), and even on micro-organisms in closed culture such as yeast (9, 10); all point to the fact that as the organism ages, part of its proteins, water, and associated minerals is progressively replaced by fat.

A dramatic illustration of this tendency is provided by recent studies on hyperphagic (hypothalamic) animals (11). The regulation

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weight increase per gram of diet, and 0.81 the efficiency at weaning. After 30 days, this efficiency has fallen to 0.31, and after 70 days, to 0.17. If, however, the efficiency is calculated in terms of calories of new tissue by calories ingested, the initial value is found to be 0.35; after 70 days, when the animals weigh on the average 385 grams, this efficiency would still be 0.35 if 40% of the weight increase at that time was constituted by fat. In fact, it has decreased but relatively little. If this "energetic" efficiency is represented by an equation analogous to (II),

$$E_t^1 = 0.35 - B \log t \qquad \text{(IIa)}$$

B is at the most in the order of 0.08, this value corresponding to a proportion of 20% of the daily weight increase of a 385 gram rat constituted by fat.

of food intake is disrupted by the lesion with resultant increase of appetite, and the animal becomes increasingly fat. All biochemical observers agree that the lesion causes no fundamental disruption of the metabolic pathways. The efficiency of food conversion to body tissue is not modified, but because the whole picture of metabolism is oriented toward fat synthesis in adult animals, the animal lays down fat rather than proteins.

Very different is the picture obtained under the influence of injections of growth hormone. There, one observes a return of youthful characteristics so that the picture is one of protein retention or synthesis rather than the normal adult picture of nitrogen equilibrium and fat synthesis (12).

The same interpretation might perhaps be extended to the energetics of tumor tissue. It may well be that there is no fundamental alteration in the over-all synthetic capacity of cancerous cells, but that there is a sudden or a progressive reversal from the adult characteristic of fat synthesis to the youthful characteristic of protein synthesis, similar to that demonstrated by cultures of isolated cells or tissues of multi-cellular organisms. That these cells or tissues are "potentially immortal" may be interpreted as showing that if certain endocrinological influences of the organism are withheld, these units continue in the pattern of protein synthesis and are not submitted to the process of ageing. Similarly certain micro-organisms reproduce or accumulate fat according to the extent of their exposure to the toxins secreted by a similar micro-organism in the same medium (9). Fat synthesis, or ageing processes, continue until a properly senile phase is reached where intra-cellular conditions created by fat deposition are such that no synthesis is possible.

That the efficiency of conversion of food energy to tissue energy does not drop to a zero value at adulthood has extremely important, practical connotations. In particular, a diet promoting good economical growth in youth tends to promote fat deposition in adulthood, and possibly the development of degenerative diseases and malignancies (13). The assumption that diets judged adequate in growth studies are good "maintenance" diets appears particularly dangerous in this light.

Certain observations which had not been properly correlated may be susceptible of becoming integrated into this general pattern. For instance, the observed beneficial effect of high levels of dietary fat in

children and adolescents, translated in greater "allowances" of dietary fat for these groups than for adults (14) can be easily interpreted if it is considered that the metabolism of the young organism is such that it has a very limited capacity of fat synthesis. Similarly, this theory would interpret the fact that of all three main classes of foodstuffs, fats possess the smallest specific dynamic action in "adult" (e.g., "ageing") organism (15).

Summary

1. Ageing is defined not essentially by a decrease of the efficiency of food utilization for synthesis, but by a re-orientation of this synthetic ability to fat rather than to protein.

2. Ageing can thus be expressed quantitatively by the ratio:

$$a_t = \left(\frac{\partial W_f}{\partial W}\right)_t$$

where W is the total energy content of the organism at the time t, and W_f the part of the energy content due to fat.

3. Various implications of this definition are considered.

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THE LEUCOCYTES IN REGENERATING LIMBS OF TRITURUS VIRIDESCENS

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It is frequently maintained that leucocytes are instrumental in regenerative processes of urodele Amphibia. Yet despite the great amount of research conducted in this field, there are only a few studies on the role of the white blood-cells in regeneration. Most of these are antiquated and often confused, a situation which seems to justify reinvestigation.

Colucci (1886, quoted from Fritsch), who was first to examine in detail the histology of regeneration in urodeles, maintains that the regeneration bud (blastema) of the adult is formed by leucocytes originating from the blood-vessels and bone marrow, an opinion later rejected by Marchand (1901) and others. Wendelstadt (1904) observed, in adults, infiltration into the wound of small cells of unknown origin and their multiplication here. These elements, singly or fused into giant-cells, are claimed to resorb the damaged bone or cartilage; he also maintains that they contribute to the restitution of the connective tissue. Fritsch (1911) stresses the fact that the elements concerned with the resorption of the bone are leucocytes with simple round nuclei whilst the blastema is formed by cells of local origin, of an unknown nature.

The only investigation of consequence which differentiates between the various types of leucocytes is that of Hellmich (1930) who studied urodele and anuran species of different ages. He found fourteen kinds of hemato- and histiogenic migratory cells, several merely representing slight variants of the same type. To some of them he tries to ascribe a function. Of those which concern us here the neutrophils are stated to have a fermentative-defensive function, presumably against invading bacteria. The activity of the eosinophils is simi-

[1]The greater part of the present study was completed at the Department of Biology, Princeton University. It was aided by a grant from the John and Mary R. Markle Foundation, for which grateful acknowledgment is herewith expressed.

larly concerned with the latter. Some of the lymphocytes are assumed to phagocytose injured or necrotic elements and the macrophages (his polyblasts) to be phagocytic and also to contribute to the new tissue. The whole problem is obscured by the fact that he establishes several unreal cell types and his conclusions regarding the function of the leucocytes are based on few actual observations.

Material and Methods

An attempt to investigate conditions in larvae had to be given up as their leucocytes were found to be largely composed of unspecific "blast" cells. Subsequently only mature individuals were studied.

Twenty-six specimens, males and females in equal proportion, ranging in size from 7-9.5 cm. were used. Of the total number, 19 were operated on, on April 7th, the remainder on November 16th. Chloretone ($1^{0/00}$ aqueous solution) was used as anesthetic. Amputation was performed through the middle of the forearm of the front-leg by a transverse cut with iridectomy scissors. Several minutes after amputation, protruding parts of the bones were trimmed level with the wound surface, a procedure which ensures a more uniform course of regeneration. After the operation all individuals were kept in the same aquarium at room temperature. No complication nor losses occurred.

At intervals ranging from 6 hours to 16 weeks post-operation, the limbs were removed at the shoulder and fixed in Zenker-formol. For decalcification 3% aqueous formic acid proved far superior to several other liquids tried. Serially cut paraffin sections (6-10 μ) were stained with Wright, Mallory, hematoxylin-eosin and basic thionine in the mentioned order of frequency. Blood smears were studied in addition, to gain information on the circulating hemocytes.

Observations

The present investigation reveals the presence of two distinct though overlapping phases in the activity of leucocytes in the operated limbs: (a) a catabolic phase characterized by phagocytic, lytic and eliminatory processes and (b) an anabolic phase distinguished by the release by the leucocytes of substances which apparently cause growth of the regenerate. In the first, neutrophils, eosinophils and macrophages are involved in order of sequence; in the second, mast-

cells and lymphocytes. The subject is accordingly presented in the same order. Of the non-hematoid elements and tissues, only those will be considered whose condition reveals a connection with, or dependence on the leucocyte activity. A separate section deals with the formation of the blastema and its relation to leucocyte function.

1. The Reaction of the Leucocytes

Neutrophils (*Special Leucocytes*). An infiltration of neutrophils into the wound is already noticeable 6 hours after the operation. Occasionally diapedesis through the walls of adjacent blood vessels is observed suggesting the origin of these elements. Phagocytosis is rather rare at this stage.

There are also numerous erythrocytes in the wound at this time, due undoubtedly to the extensive hemorrhages caused by the amputation. The occasional occurrence of a basophil, lymphocyte or eosinophil is probably also due to the same cause.

Twelve hours after operation the number of neutrophils reaches a high level and remains so during the 24-hour stage. This is due to a further influx suggested by the numerous diapedeses through the blood vessels and the presence of healthy young elements. The infiltration, earlier limited to the wound, now extends in a lessening degree halfway to the elbow. A blockage of capillaries is also noticeable. Some neutrophils now contain phagocytosed cell fragments, nuclei or pigment. Ingested whole cells are rare and no phagocytosis of erythrocytes was observed. The latter succumb to gradual disintegration, only a small part of the fragments being phagocytosed. The neutrophils, most frequently appear to surround and ingest muscle fragments. Occasionally a neutrophil contains non-staining vacuoles which may suggest an uptake of liquid or semi-liquid material (pinocytosis).

Neutrophils are also observed in what appears as intact muscle or bone. It is, however, very doubtful whether phagocytosis of intact tissue takes place to any marked extent. Such an activity would find expression in a noticeable involution of the amputated arm; involution, however, is minimal and limited to the injured area. As to the bone, there may be a limited lytic action of the leucocytes and other elements; this is suggested by the observation that its cut ends, sharp and rugged at first, begin to round off 2-3 weeks after amputation.

Already at the 24-hour stage a few of the neutrophils within the wound appear to disintegrate, a process which is well pronounced 48 hours post-operation. In the latter phase there is also a marked decrease in their number, but the proportion of those containing ingested débris is larger than before. A further decrease of the elements under consideration is observed at the 3½ days' stage; at this time, they are mostly found in and around the wound. One week after the amputation their number is usually still somewhat elevated but reaches a normal and, within the wound proper, a subnormal level two weeks post-operation, when their function appears to be definitely terminated.

There is no evidence to connect the presence and function of the neutrophils with bacterial invasion, as surmised by Hellmich and others. Although our operations were not performed under aseptic conditions, no signs of infections were observed and the wounds were usually overgrown with new epidermis 12 hours post-operation, occasionally even earlier. Experience shows that in Amphibia, as in poikilothermous animals in general, wound infections are much rarer than in warm-blooded forms.

As to the ultimate fate of the neutrophils in the affected area, there is no evidence that they return into circulation. All signs lead to the conclusion that, having performed their function, they soon die. Besides those which disintegrate in the wound, additional ones laden with débris and pigment migrate into the new epidermis covering the cut. The process is already on its way 12 hours post-operation. In the epidermis they show pycnotic nuclei and are evidently in a dying condition (Fig. 1). They or their remnants are apparently soon eliminated as suggested by their almost complete absence from the epidermis one week after the operation. Under normal conditions, the epidermis contains only lymphocytes.

Eosinophils. Eosinophils appear somewhat later in the wound than the neutrophils, viz. 12 hours post-operation and remain relatively abundant during the 24-hour stage. Subsequently their numbers gradually decline and reach a normal level 2-3½ days, exceptionally only after the first week, following operation. Their origin as far as concerns the wound, has not been determined. Normally they are quite common in the circulating blood and in the connective tissue.

The function of the eosinophils manifests itself morphologically

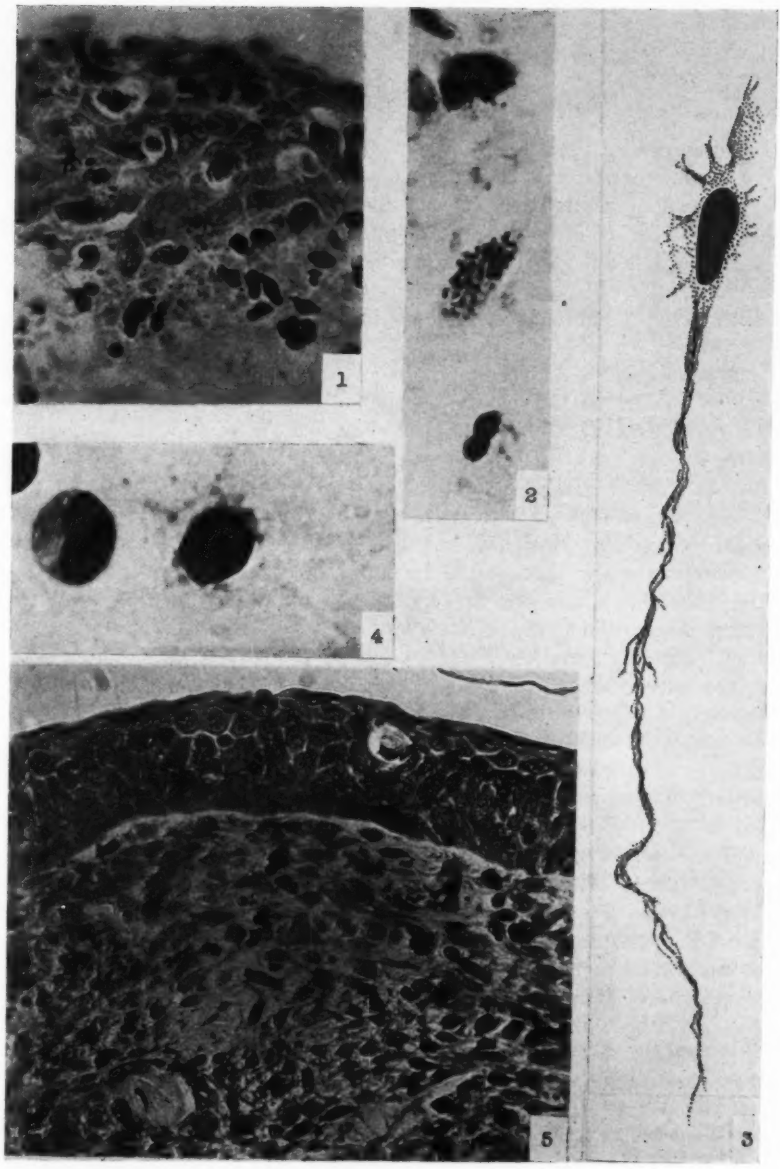


PLATE 1

Explanation of Figures

Figures 1, 2, 4, 5 are unretouched photomicrographs, Figure 3 a camera-lucida drawing. All are from sections stained with Wright's solution except Figure 4 which is from a preparation stained with basic thionine.

1. Neutrophils in and below the new epidermis; upper edge of section out of focus; 370 ×.
2. Center and top: macrophages with pigment; bottom: disintegrating lymphocyte; 660 ×.
3. Tissue mast-cell, from a single section; 400 ×.
4. Center: a lymphocyte shedding its cytoplasm; left: a macrophage; preparation is overstained to render the cytoplasmic particles visible; 1250 ×.
5. Section of a blastema, three weeks after operation; 220 ×.

by liberation of their granular inclusions, which is often accompanied by a disintegration of the cell. On the precise significance of this phenomenon, particularly its physio-chemical aspect, we are still in the dark. It is, however, the general consensus that their function is connected with plasmolytic processes, more precisely with protein breakdown. The fact that their activity coincides with the period when cellular débris is at the maximum, lends additional support to this thesis.

Macrophages. A few macrophages with ingested pigment are observed in the wound at the height of neutrophil activity (12 and 24 hours' stage). Their number, however, appears increased only after that of the neutrophils has markedly declined, i.e. 3½ days post-operation. They remain at the same, relatively high level for a long period and scattered elements are still encountered 5-6 weeks after operation. However, the intensity of their infiltration never appears to reach that of the neutrophils. Their activity, as far as the microscopic picture reveals, is phagocytic, pigmented material being most often found in their cell bodies (Fig. 2). In this respect they probably complete the function of the neutrophils. Their late appearance suggests a tardy response and their long persistence possibly a slower action than those of the neutrophils. They seem to return to the circulation as is indicated by the occasional encounter of an element with ingested material far off the wound. Some, heavily loaded with ingested pigment, apparently change into pigment cells.

Their origin as far as could be ascertained is histiogenic; they appear to develop locally from fibroblasts as the latter retract their long filaments, round up and become ameboid. Occasionally an element is encountered which bears some semblance to a large lymphocyte, but there is no evidence that the latter change in significant numbers—if at all—into macrophages.

Mast-cells.[2] There are very few tissue mast-cells in the limbs of the species studied; they are large with very long, branching processes (Fig. 3). The latter are extremely abundant in the connective tissue, especially that of the hypodermis, suggesting that the

[2]This term is applied here to the basophilic elements of both the tissues and the blood, as the two kinds are known to be genetically related in the lower vertebrates. The far more flexible term "mastocyte" should be introduced as it lends itself to form composite words, e.g., mastocytopenia, hemomastocyte, etc.

mass of the processes may well exceed that of the cell proper. Granules and protoplasm are known to move in these processes.

Six hours after amputation the mast-cell processes in and around the wound appear broken and curled up or changed into droplets. At the 12 hours' stage this change seems to have extended half-way towards the elbow and persists through the 24 and 48-hour stages. Three and a half days post-operation the number of droplets resulting from the disintegration of the mast-cell processes has greatly diminished near the wound and this decrease extends over the whole lower arm one week after operation. A return to normal is noticed only 4-5 weeks after amputation.

There is no indication that the mast-cells of the blood (mast-leucocytes, basophils) in any way react during the regeneration process. They appear, however, to replenish the tissue mast-cells, after the latter have become exhausted.

Lymphocytes. Lymphocytes, originating both from the blood and the tissues, begin to appear in increased numbers in the vicinity of the wound 3½ days after the amputation. At the one-week stage they have penetrated into the wound proper and generally are increased in number; after another week they dominate the picture and the infiltration affects two-thirds of the lower arm, including the microscopically discernible beginnings of the blastema.

There is a noticeable decrease in the number of the lymphocytes and their activity three weeks after the amputation. This trend continues for another two weeks. At the end of this period their number is practically normal except for the tip of the blastema. At this time the first signs of tissue differentiation in the blastema become apparent.

The infiltration of the lymphocytes is paralleled by disintegration of these cells (Fig. 2) or their giving off cytoplasmic particles (Fig. 4). In the first and more frequent case the lymphocytes often appear as more or less homogeneous, strongly basophilic masses before breaking up into several pieces which in turn appear to dissolve; there are also pictures suggesting a direct dissolution of the lymphocytes. In the less common cases, the nucleus appears normal, the cell merely giving off cytoplasmic particles. No phagocytosis was observed in the lymphocytes; divisions were not uncommon amongst them.

2. The Origin, Composition and Growth of the Blastema

The involutionary processes connected with the action of the neutrophils, eosinophils and macrophages were described in the sections dealing with these elements. The growth of the regenerate, or more precisely that of the blastema, can be attributed to the activity of the mast-cells and lymphocytes and is dealt with in the following sections.

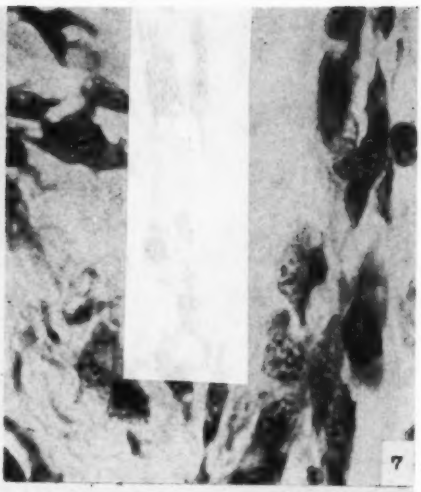
Our observations indicate that the blastema is composed almost exclusively of fibroblasts and the epidermis forming its cover (Fig. 5). Hence a study of these two components, their origin, growth, migration, etc., in addition to suggesting a possible effect on them of the lymphocytes and mast-cells, helps to elucidate the initial processes of regeneration generally associated with blastema formation.

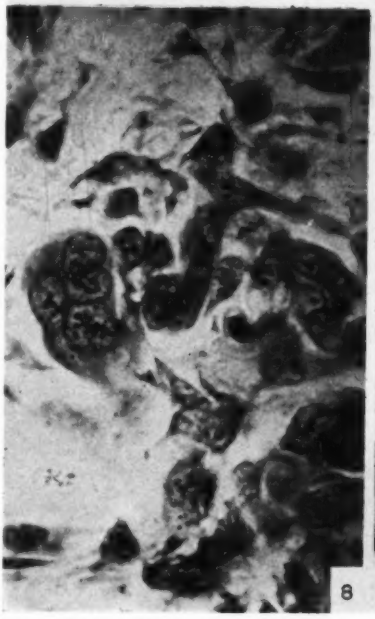
The fibroblasts. One or two mitoses of fibroblasts are observed in the injured area 3½ days post-operation, becoming quite common on the seventh day. At the two weeks' stage their number reaches a peak, the mitotic activity having spread almost to the elbow. There is a marked decrease in mitoses a week later, followed by a further reduction of their number in the fourth week. Only an occasional division is detectable in the 5 and 6 weeks' stage.

A comparison with the rate of infiltration and fragmentation of the lymphocytes reveals a close parallelism between the latter and the intensity of mitotic activity of the fibroblast. In addition, it should be added that the mitotic wave is also preceded by the disappearance of the mast-cell products from the affected area.

Apparently due to the numerous divisions a movement of the new fibroblasts towards the wound and beyond sets in, which produces a continuously growing bulge at the cut end. This swelling is the blastema (regeneration bud). Whether the migration is active or merely caused by a "squeezing out" process due to overcrowding could not be determined from our material; it should, however, be noted that fibroblasts are known to move by a peculiar gliding or streaming motion.

In addition to the mitotic activity, there take place in the fibroblasts changes in the cytoplasm. Normally the fibroblasts show very little cytoplasm, often only the characteristic nucleus being discernible (Fig. 6). Preceding and parallel to the mitotic activity of these cells, a considerable increase of the amount of cytoplasm takes place





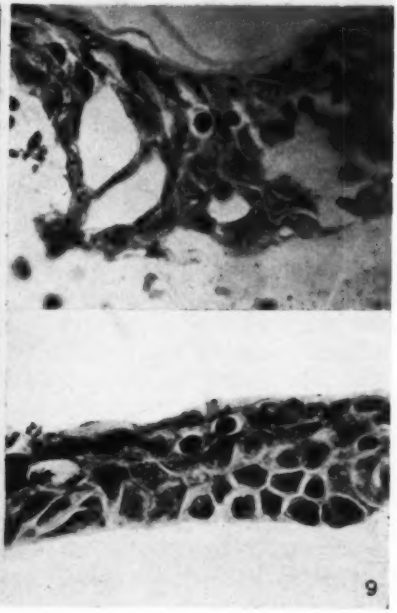


PLATE 2

Explanation of Figures

All figures are unretouched photomicrographs from sections stained with Wright's solution.

6. Fibroblasts of a regenerating leg in a region removed from the blastema; 570 ×.
7. Fibroblasts in the vicinity of the blastema, one of them in mitosis; same section as Fig. 6; 500 ×.
8. Giant cells in a regenerating leg, three weeks after amputation; 500 ×.
9. New epidermis covering the wound; above: 6 hours, below: 12 hours after amputation; black spots are pycnotic lymphocytes; at the left of lower section: an epidermal cell in mitosis; ca. 300 ×.

(Fig. 7). The latter assumes a homogeneous, mat, appearance; but some preparations suggest the presence of a dense reticulum of extremely fine fibrils. The cytoplasm is distinctly basophilic and metachromatic, suggesting the presence of a ribonucleotide and high molecular ester sulphuric acid.

Considering that basophilic and metachromatic substances are released by the lymphocytes and mast-cells during the growth phase, the question arose whether a direct uptake of these substances by the fibroblasts could be detected. Whilst there is no evidence that lymphocyte products are taken up, there are indications that this may be the case with the mast-cells. Their processes are in very close contact with the fibroblasts, as with other cells, and frequently surround them with a network. Some pictures suggest that these processes may actually extend into, or coalesce with, the cytoplasm of other cells; the methods applied in the present study were, however, not fine enough to render a conclusive opinion on this point. However, the absence of a microscopically detectable uptake does not preclude the possibility of indirect utilization by the multiplying fibroblasts and other cells of substances derived from the leucocytes.

According to our observations, the so-called "giant cells" (Fig. 8) also belong to the category of fibroblasts. They first appear 2 weeks post-operation on the surface of the amputated bone. Here the fibroblasts are very numerous and closely packed and it is often impossible to decide whether a case represents a dense aggregation or a syncytium of these elements, a giant cell. There are pictures on the cut bone surfaces suggesting that elements originating from the marrow and undistinguishable from lymphocytes also enter into formation of giant cells; in the completed form, however, they do not differ from the young fibroblasts, either in nuclear or in cytoplasmic structure.

Concerning the function of the giant cells, no phagocytosis could be discerned in them as assumed by some earlier investigators. In later phases, when proliferation subsides (5th-6th week), they become rare and small, part of them having formed several mesodermal structures, another possibly dissociating into individual fibroblasts and like them making up the as yet undifferentiated portion of the blastema.

The epidermal cells. Within 12-24 hours after the amputation, the

wound becomes covered by epidermal cells. These are elements from the stump which move over and close the wound. In this process many of them apparently become de-differentiated, assuming an ameboid or fibroblast-like form (Fig. 9). At the 24 hours' stage, occasionally even 12 hours after operation, a few mitoses become apparent in the portion covering the cut and its immediate vicinity, but remain scarce during the 2 and 3½ days' stage. A high peak is only reached 1-2 weeks after operation. It appears to subside in the third week and drops sharply in the fourth. Only an occasional mitosis is encountered 5 weeks after operation.

A comparison with the mitotic wave pattern of the fibroblasts reveals a coincidence of their peak of activity (about 2 weeks after operation) and a probable dependence of both on the dissolution process of the lymphocytes which shows a maximum during the same period. In the epidermis, however, the high of mitotic activity is preceded by a low which sets in 12-24 hours after amputation. This low wave can also be connected with the activity of lymphocytes. Normally the epidermis contains a great number of these cells; soon after the operation those in the portion of the epidermis which moves over the wound become pycnotic and disintegrate (Fig. 9) and it is feasible that the early mitotic activity is caused or made possible by the release of lymphocyte substances. The mast-cells, whose processes are most common in the hypodermis and which begin to disintegrate at the time of the first mitoses in the epidermis, may also be in part connected with this process, and the appearance of metachromatic material in the cells of the new epiderm lends some support to such a view.

Discussion

The sequence and reaction of the leucocytes following amputation in *Triturus* correspond in the main to those found generally among vertebrates.

While there is not much that can be added to our earlier knowledge on the role of the neutrophils and macrophages (cf. Menkin, 1940) and the eosinophils (Ringoen, 1938; Kirk, 1942), considerable information has recently accumulated on the nature and function of the elements active during the growth process: the mast-cells and lymphocytes.

The mast-cells, as the name implies, were early assumed to be

connected with the nutritional condition, a view which, though repeatedly substantiated, became obscured by the persistent tendency to attribute phagocytic, eliminatory or defensive functions to *all* leucocytes (Michels, 1938). The close morphological, cyto-chemical and functional similarity of the mast-cells with the trephocytes of the invertebrates has been demonstrated recently (Liebman, 1946, 1947). Like the trephocytes, they are non-phagocytic, a quality conceded even by those who deny a trophic function to the mast-cells, and poorly or non-ameboid; the substances contained in their cytoplasm are essentially of a trophic nature and are emitted into the surrounding. The observations of Brodersen (1928) suggest that in the mouse they pass directly to the fibroblasts; this too seems to be the case in *Triturus*. Direct passage of trephocyte products, or intact elements, into other cells has repeatedly been observed in invertebrates (Liebman, 1946, 1947). Regarding the relation of the mast-cells to the fibroblasts and epithelial cells in mammals, Sylvén (1938, 1941) came to conclusions strikingly similar to the present ones of the writer: following injuries, metachromatic substance appeared in the fibroblasts and epidermal cells after it disappeared or diminished in the surrounding mast-cells. He, as well as Holmgren (1939, 1940), stress that certain kinds of metachromasia characterize growing and embryonic tissues. Contrary to earlier beliefs, Sylvén (1945) further claims that the mast-cells and the metachromatic inclusions liberated by them have a growth-promoting role in certain tumors. It should be added that the basophilia of the mast-cell granules indicates the presence of ribonucleic acid (Caspersson and Schultz, 1939, 1940), a substance essential to protein synthesis and growth in general.

It has repeatedly been conjectured that the lymphocytes have a trophic function. Loreti (1934, 1935) interpreted their presence and dissolution in the intestinal and renal epithelium of fishes and Amphibia as representing a nutritive process. Andrew and associates (1945, 1946, 1947), who observed them in the epithelium of the digestive and respiratory tract, assume that they perform a defensive function here. But this view does not necessarily conflict with the previous one if the defensive action is achieved by the liberation of antibodies. White and Dougherty (1946 *et ante*) have demonstrated that the lymphocytes of mammals contain and liberate γ and β globulins and antibodies are assumed to be modified globulins. The

penetration of lymphocytes into the ovarial eggs of *Triturus* during the growth period and their disintegration within them (Liebman, 1945) is hardly explainable along other than nutritive lines. Neither is it permissible to limit the number of substances released upon disintegration to globulins: the strong basophilia preceding this process indicates the presence of ribonucleic acid and the nuclei, which make up a considerable part of the lymphocyte bodies, in addition contain desoxyribonucleic acid.

There is considerable uncertainty and confusion regarding the nature of the main cellular components of the blastema. The views of earlier investigators mentioned in the introduction were for some time replaced by the notion that they are "regeneration cells" derived largely from mesenchyme elements but also from osteoclasts, polyblasts or fibrocytes (Hellmich, 1930). More recently the view has been widely established that they represent completely dedifferentiated cells arising locally from the existing differentiated elements (Schotté, 1939; Rose, 1948).

The present study suggests that the cells making up the blastema proper (its core) are fibroblasts derived from similar elements of the stump. What takes place in them prior and during blastema formation, and is sometimes taken for dedifferentiation, actually represents activation and growth; the increase and peculiar changes in the cytoplasm, the enlargement of the nucleus and its mitotic activity, and finally the division of the cell hardly permit a different interpretation. During this process the fibroblasts admittedly often assume irregular or ameboid forms which are regarded by some as signs of dedifferentiation. It is, however, doubtful whether in the present case such a conclusion is justified, as the fibroblasts actually are non-differentiated, pluripotent elements as indicated, *inter alia,* by their change into macrophages and other elements during the first, post-operative phase. Fibroblasts are apparently common to all vertebrates and the processes taking place in them prior to blastema formation in *Triturus* are essentially similar to those found in mammals during the formation of the granulation-tissue in wounds (cf. Sylvén, 1941).

There is no evidence in our material that the epidermis contributes to the blastema proper, a claim first made by Godlewski (1928) and recently revived by Rose (1948). Unlike the fibroblasts, the epi-

dermal cells do dedifferentiate, in that they lose their epithelial shape and become irregular, ameboid and occasionally similar to fibroblasts, but nothing has been observed to suggest that they migrate towards the core of the blastema and become part of the fibroblast mass as claimed by these writers. Migration of epidermal cells appears to be of general occurrence amongst vertebrates, as a means of wound occlusion. A similar process has repeatedly been observed in invertebrates, suggesting that it may be a universal mechanism of closing body surfaces.

The increased activity of the mast-cells and lymphocytes, and the leucocytes in general, returns to normal 5-6 weeks post-operation. At this time the first morphogenetic processes become microscopically discernible in the blastema, but there is no evidence of any specific or extraordinary activity of the leucocytes during this, the phase of regeneration proper. Nonetheless, as it is generally agreed that failure of blastema formation leads to lack of regeneration, it is conceivable that a reduced activity or functional failure of the leucocytes, specifically the lymphocytes and mast-cells, may inhibit formation of a blastema and thus, indirectly, regeneration.

Summary

The present investigation reveals the existence of two distinctive though overlapping periods in the activity of leucocytes in the region of amputation in *Triturus*: (1) a catabolic phase with phagocytic-eliminatory processes predominating, and (2) an anabolic phase during which leucocytes liberate substances apparently having growth-promoting qualities. In the first, neutrophils, eosinophils and macrophages become active in the order of succession mentioned; in the second, mast-cells and lymphocytes. The origin of these elements relative to the regenerating region is traced.

The neutrophils and macrophages ingest elements damaged by the cut. There is no evidence that intact tissue is phagocytosed by these cells nor that their activity is elicited by invading bacteria. The neutrophils containing phagocytosed material appear to be short-lived; some of them disintegrate in the wound, others are eliminated through the new epidermis. The macrophages do not appear to succumb to this fate. The reaction of the eosinophils is believed to be concerned with the proteolytic processes taking place during the period under discussion.

The activity of the mast-cells and lymphocytes manifests itself by release of cytoplasmic particles and inclusions; in the course of this process a considerable fraction of the lymphocytes disintegrates or dissolves. Following and concurrently with these processes, and apparently elicited by them, mitotic divisions set in amongst the neighboring fibroblasts and epidermal cells, leading ultimately to the formation of a blastema.

The blastema was found to be composed almost exclusively of fibroblasts bounded by epidermal cells. Each of these components arises through proliferation and migration of corresponding elements in the stump. No evidence was found that the epidermal cells change into fibroblasts nor that both originate from highly differentiated elements. The giant cells appear to represent syncytia of fibroblasts with similar developmental potencies; no phagocytosis was observed in them.

The increased activity of the leucocytes in and around the amputation region ceases 5-6 weeks after operation. At this time the first signs of morphogenetic processes become microscopically discernible in the blastema, but there is no evidence of a specific or increased activity of the leucocytes during the ensuing morphogenetic period. This, however, does not preclude the possibility of an indirect effect of the leucocytes upon this phase of regeneration.

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THE EFFECT ON EMBRYOS AND YOUNG OF RAINBOW TROUT FROM EXPOSING THE PARENT FISH TO X-RAYS*

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This experiment was undertaken to determine the effect of X-radiation upon spawning and subsequently upon the offspring of adult rainbow trout, *Salmo gairdnerii* Richardson, which were approaching sexual maturity. An evaluation was made of the effects of different dosages of X-radiation administered to parent fish on the survival, teratogeny, and growth of the progeny. A report on the irradiated parent fish will appear separately. In the experiments recently reported upon by Bonham, *et al.* (1948) and Welander, *et al.* (1948), chinook salmon were used as the test animals. In this study trout were used in preference to salmon since they matured at an earlier age and could be held in fresh water ponds under observation through a complete life cycle.

Materials and Methods

The trout used in this experiment were part of one generation of a stock which had been selectively bred for many generations. While they were not the progeny of a single set of parents, their heritage was similar and the individuals had become thoroughly mixed through confinement in the same pond. When this study began during the later part of December 1944 the fish were approximately twenty months old, and were approaching sexual maturity. They averaged 39 cms. in length.

The adult fish were segregated into a control group and seven groups which were to be treated. Total body doses of 50 r, 100 r, 500 r, 750 r, 1000 r, 1500 r, and 2500 r were administered at a rate of 8.25 r per minute. Twenty fish were exposed in each group except for the 1500 r and 2500 r doses where sixteen and twelve fish, respec-

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tively, were treated. Four fish were exposed at a time by holding them in a canvas jacket anchored in a plywood tank which was continually supplied with fresh water. The fish were located in the X-ray beam forty-eight inches from the target. The generator, a Picker-Waite therapy unit, was operated at a potential of 200 KV with a current of 20 MA. In addition to the filtration inherent in the oil cooled tube and in the intensimeter, filters of 0.5 mm. of copper and 1.0 mm. of aluminum were used. The actual intensity of the radiation received by the fish was determined by a Victoreen condenser r-meter. So that each side of the fish would receive the same dose, the animals were turned to face in the opposite direction when the exposure was half completed. At the same time a correction was made for the somewhat greater intensity near the center of the beam than at the edges by moving the fish in the two center compartments to the outer compartments, and vice versa.

Immediately following exposure to the X-rays the fish were measured, certain fins were removed and tags were attached for future identification. The tags used consisted of two small circular discs of celluloid which were fastened one on each side of the back of the fish near the base of the dorsal fin by a nickel-coated pin inserted through the dorsal musculature. The tags were numbered so that each individual could be identified. Furthermore, the tags were colored either white, yellow, green, or red in order that particular combinations of these colors could be used to designate particular doses. The removal of one or two fins was used as a means of identification also.

Following exposure to the X-rays, tagging, and marking, the trout were held in a large rearing pond and given routine feeding and care. The first three females were spawned by the common stripping process during the first week of February 1945. Others were spawned as they reach maturity during the next nine weeks. The eggs of each female were fertilized with the sperm of a male which had received a like exposure to X-rays and were thereafter incubated as a separate lot.

Soon after the eggs were fertilized they were placed in a drip incubator. On the second or third day the lots were examined and the infertile and dead eggs which could be detected were removed. With the closure of the blastopore, the eggs became comparatively resistant to handling which permitted frequent inspection and removal of dead eggs.

Shortly before the eggs were expected to hatch the trays were removed from the drip incubator and placed in hatchery troughs with running water. The hatching period extended from the latter part of March until the first part of May.

The fry lots were frequently inspected and a record was kept of the mortality. In addition, all obviously abnormal fry were removed and classified. Soon after the oldest fry had completed absorption of their yolk and had started to feed, the small compartments in the troughs to which they were confined became crowded, necessitating the combining of offspring from parents which had received the same amount of X-radiation. With continued growth, repeated reductions were made in the number of fish under observation to prevent overcrowding. By the middle of August it was necessary to transfer the fish, now of fingerling size, from the hatchery troughs to outside ponds.

Results

Gametogenesis

The eggs and sperm produced by the adult trout were partially mature at the time the parent fish were X-rayed and probably were not derived from oocytes or spermatocytes developed following treatment. Thus the maturing germ cells also received the X-radiation. Since spawning extended over a considerable period of time, the possibility was investigated that some recovery of the germ cells may have taken place, or else that cells in earlier stages of maturation might have been more or less severely damaged by the X-rays. In Table I the number of days between exposure and spawning has been tabulated for each dose together with the resulting egg mortality. Males and females are listed separately but each complements the other since each egg lot represents the progeny of one male and one female. Variations occurred in each grouping but there seemed to be no relationship between egg mortality and the time elapsed between treatment and spawning. If recovery of injured germ cells did take place or if some stages were more sensitive to X-rays than others, such facts could not be demonstrated from the data and were obscured by the wide variations. Recovery of the gonads of *Oryzias* from the effects of X-radiation reported by Solberg (1936) is not strictly comparable since in his fish recovery involved using the injured germ cells and subsequent production of normal ones.

TABLE I

MORTALITY OF RAINBOW TROUT EGGS SPAWNED AT INCREASING LENGTHS OF TIME AFTER TREATMENT OF PARENTS

Parents Exposed to 50 r				Parents Exposed to 100 r				Parents Exposed to 500 r			
Females		Males		Females		Males		Females		Males	
No. of Days[1]	Per Cent Mortality[2]	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality
21	54.9	21	54.9	30	66.8	30	66.8	38	86.3	39	86.3
55	43.4	55	43.4	38	83.5	38	83.5	51	79.5	49	95.5
55	89.6	58	89.5	52	52.2	49	79.2	52	78.7	51	79.5
60	71.5	60	71.5	55	35.3	52	52.2	52	95.5	52	78.7
62	66.7	60	89.6	56	79.2	60	35.3	54	88.5	55	73.6
63	89.5	64	68.8	58	76.5	60	46.7	55	91.0	55	91.0
69	65.5	67	65.5	60	46.7	65	76.5	56	73.6	60	88.5
71	68.8	69	66.7	67	47.0	74	47.0	74	86.6	70	86.6
77	18.3	77	18.3					82	83.6	86	83.6

Parents Exposed to 750 r				Parents Exposed to 1000 r				Parents Exposed to 1500 r			
Females		Males		Females		Males		Females		Males	
No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality	No. of Days	Per Cent Mortality
58	99.2	60	94.0	22	97.7	24	97.7	22	99.5	22	99.5
60	94.0	60	96.2	22	100	26	97.6	63	100	60	100
62	99.6	60	99.2	29	97.6	27	100	63	100	63	100
63	99.3	62	99.6	38	97.6	35	97.6	66	99.9	68	99.9
64	95.1	63	99.3	50	99.2	50	99.2	68	99.2	72	99.2
65	96.2	66	96.5	56	99.1	53	99.1	75	100	74	100
68	96.5	69	95.1	58	95.0	56	98.5	78	100	76	100
69	97.4	69	97.4	60	98.5	58	95.0	80	100	77	100
76	95.8	76	93.1	60	99.8	62	99.8				
78	93.1	78	95.8	71	98.9	83	98.9				

Mortalities on the eggs from control fish are not shown on this table since the parent fish were not treated. None of the eggs obtained from parent fish exposed to 2500 r survived.

[1]Number of days is the time between treatment and spawning.
[2]Per cent mortality is that of the eggs of the individual fish.

TABLE II

RELATIVE TIME OF SPAWNING OF FEMALE RAINBOW TROUT EXPOSED TO VARIOUS AMOUNTS OF X-RAYS

Control Fish		Fish Exposed to 50 r		Fish Exposed to 100 r		Fish Exposed to 500 r	
Tag No.	Day of Spawning	Tag No.	Day of Spawning	Tag No.	Day of Spawning	Tag No.	Day of Spawning
11000	0	10972	2	C8	4	C585	13
11013	0	10968	34	C12	17	P5738	17
10915	0	C1368	34	C3	30	P4770	25
10969	4	P4884	34	C7	34	P3473	34
11091	8	11037	37	C10	34	C20	34
11099	31	C1276	41	P4773	37	C17	37
11070	37	10980	43	C15	39	P5716	37
110963	37	P5676	45	C13	48	P4918	39
F1	48	10930	62			C397	48
						P4808	60
$\bar{x}$ = 18.3		$\bar{x}$ = 36.9		$\bar{x}$ = 30.4		$\bar{x}$ = 34.4	
t = 2.31*		t = .66		t = .38		t = .27	

Fish Exposed to 750 r		Fish Exposed to 1000 r		Fish Exposed to 1500 r		Fish Exposed to 2500 r	
Tag No.	Day of Spawning	Tag No.	Day of Spawning	Tag No.	Day of Spawning	Tag No.	Day of Spawning
11009	37	11012	2	11074	4	P9X	8
11032	41	11087	4	11010	39	P39X	8
11038	43	11054	4	11051	43	P20X	30
11031	45	11094	13	11044	48		
11022	45	11019	30	11048	48		
11020	45	11093	37	11082	53		
11057	45	11004	37	11002	56		
11056	48	11058	37	11055	56		
11092	56	11023	43				
11086	56	11011	56				
$\bar{x}$ = 46.1		$\bar{x}$ = 26.3		$\bar{x}$ = 43.4		$\bar{x}$ = 15.3	
t = 2.37*		t = 1.09		t = 1.61		t = 1.71	

0 Day is February 6, 1945, the date on which the first fish spawned.
$\bar{x}$ for all females combined is 32.8.
*Indicates values which are significantly different from mean of all females.

Another consideration was that the X-ray treatment might have accelerated or retarded the process of maturing the eggs, thus hastening or delaying the spawning date. In Table II the time of spawning of each female in each experimental group has been tabulated. Although there was an interval of sixty-two days between the time of spawning the first and last female, wide variation occurred in all groups. The mean spawning time, $\bar{x}$, of each of the experimental groups was compared with that of all females combined by the use of "Student's" t test, Snedecor (1946: 82), to see if significant differences occurred. The mean of all the females combined was thought to be a better estimate of the normal spawning time than the mean of the control group alone since some selection of fish was made for the control group in order to perpetuate the characteristics of the stock for other (selective breeding) experiments.

Spawning was found to have occurred significantly early in the control group and significantly late in the 750 r group. Other means did not differ significantly. The earlier spawning in the control group was possibly due to the selection of the fish while the late spawning in the 750 r group was possibly due to a mischance of sampling since the other means did not suggest a trend in the data.

The 2500 r group is of especial interest: Although it had the earliest mean for spawning time, it did not significantly differ from the general mean because of the small number of females involved. Two other females in this group which normally would have spawned died before the eggs were fully matured. The three females in this group which did spawn died shortly afterwards. At the time of spawning blood and serous fluid were extruded with their eggs.

These data do not give conclusive evidence that the X-ray treatments either delayed or hastened spawning. In the 2500 r group, however, some fish were so extensively damaged that they died before they could spawn.

Egg Development

The eggs which were obtained from the X-rayed trout were incubated according to standard hatchery practices. Eggs which were infertile or dead could be recognized sooner or later by an opaque white color resulting from the precipitation of the normally translucent yolk material. Such eggs were removed and nearly all of

them examined to determine whether or not an embryo was present, and if so, how far development had progressed. The eggs which were examined were classified into the following stages of development:

A. Eggs which were infertile or in which no embryonic tissue was visible.
B. Eggs in which the embryo was recognizable as a multicellular blastoderm.
C. Eggs in which the embryonic axis had differentiated from the embryonic shield and in which the blastoderm had overgrown one-quarter or more of the yolk.
D. Eggs in which the blastopore had closed completely and the embryo extended from one-third to one-half way around the circumference of the yolk sphere.
E. Eggs in which the eyes of the embryo had become pigmented and fin rays had appeared.
F. Eggs in which the embryo failed to survive the process of hatching.

A distinct direct relationship was found between the amount of irradiation administered to the parents and the subsequent mortality among their eggs (Figure 1). Further, as the amount of radiation received by the parents increased the degree of development attained by the embryo decreased. Thus of the eggs obtained from parents exposed to 500 r, the number successfully forming an embryonic axis was not appreciably different from that of the controls. A relatively greater number did not succeed in closure of the blastopore and in subsequent development. In the 750 r and 1000 r parent groups, many of the eggs were not fertilized at the outset, or, at least, embryo formation did not start in many, and the number of eggs which succeeded in forming an embryonic axis and reaching further developmental stages was materially reduced. In the 1500 r group, a majority of the eggs did not start development, very few were capable of forming an embryonic axis, and practically none succeeded in hatching. Comparatively few of the eggs from parent fish exposed to 2500 r showed development, and practically none was able to form an embryonic axis, only one egg out of more than 5000 developed as far as the eyed stage.

The variations in percentage mortality among the eggs of individual females within each of the treatment groups were large, neces-

TABLE III

COMPARISON OF MORTALITIES OF EGGS OBTAINED FROM PARENTS TREATED WITH VARIOUS AMOUNTS OF X-RAYS

Eggs from Control Parents				Eggs from Parents Exposed to 50 r				Eggs from Parents Exposed to 100 r				Eggs from Parents Exposed to 500 r			
Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.
1	2093	613	29.3	5	2554	1401	54.9	6	1985	1325	66.8	17	1682	1451	86.3
2	3314	270	8.1	25	1365	593	43.4	16	1974	1649	83.5	18	1426	1133	79.5
3	2677	377	14.1	27	2266	2031	89.6	20	1415	1120	79.2	26	1671	1595	95.5
9	2342	1083	46.2	29	1188	849	71.5	23	1984	927	46.7	28	1378	1014	73.6
12	2150	1082	50.3	34	953	853	89.5	24	1537	542	35.3	30	1405	1279	91.0
22	2109	1441	68.3	44	1736	1137	65.5	37	1555	811	52.2	38	1242	977	78.7
32	1528	1318	86.3	47	2679	1786	66.7	40	1301	995	76.5	41	1755	1554	88.5
33	1526	406	26.6	51	1560	1073	68.8	58	1902	893	47.0	56	1264	1094	86.6
59	1856	1397	75.3	67	1972	360	18.3					66	1638	1369	83.6
	Mean $\bar{x}$		44.9		Mean $\bar{x}$		63.1		Mean $\bar{x}$		60.9		Mean $\bar{x}$		84.8
					t		1.53		t		1.39		t		4.19[1]

Eggs from Parents Exposed to 750 r				Eggs from Parents Exposed to 1000 r				Eggs from Parents Exposed to 1500 r				Eggs from Parents Exposed to 2500 r			
Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.	Lot No.	No. of Eggs	Total Mort.	% Mort.
39	1640	1627	99.2	4	2287	2287	100.0	7	1710	1702	99.5	11	2318	2318	100.
43	1657	1594	96.2	8	2452	2395	97.7	42	1502	1502	100.	13	2327	2327	100.
46	1994	1875	94.0	10	1847	1802	97.6	45	1742	1742	100.	19	639	639	100.
49	1409	1340	95.1	15	1897	1851	97.6	54	1746	1744	99.9				
50	1713	1706	99.6	21	1101	1092	99.2	55	1513	1501	99.2				
52	1176	1146	97.4	31	1253	1251	99.8	60	1740	1740	100.				
53	1575	1520	96.5	35	1446	1424	98.5	61	1573	1573	100.				
57	2177	2161	99.3	36	1485	1471	99.1	62	1537	1537	100.				
63	1821	1695	93.1	48	1397	1327	95.0								
65	1389	1330	95.8	64	2409	2382	98.9								
	Mean $\bar{x}$		96.6		Mean $\bar{x}$		98.3		Mean $\bar{x}$		99.8		Mean $\bar{x}$		100.
	t		5.95[1]		t		6.14[1]		t		5.60[1]		t		3.36[1]

[1]Indicates a probability of less than 1 per cent that mortalities are not different than those of the controls.

sitating statistical treatment to demonstrate the significance of the differences between the means of the several groups (Table III). The mean percentage mortalities shown are slightly different from the average mortalities in Figure 1 due to different methods of computa-

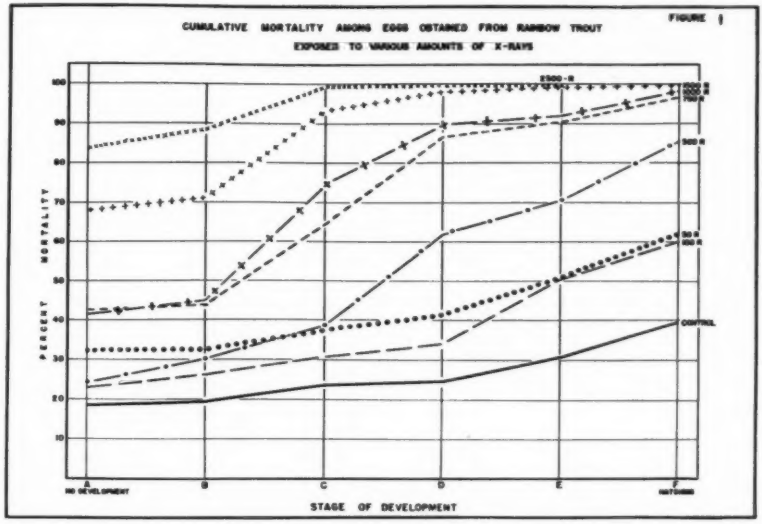

CUMULATIVE MORTALITY AMONG EGGS OBTAINED FROM RAINBOW TROUT
EXPOSED TO VARIOUS AMOUNTS OF X-RAYS
PERCENT MORTALITY
STAGE OF DEVELOPMENT
CONTROL

FIG. 1

tion. In Figure 1 the average mortality is that of all lots combined, and in Table III it is the mean of the averages of each lot. The mean percentage mortalities ranged from 44.9 in the control lots to 100 in the 2500 r lots. The mean mortalities of the eggs obtained from parents subjected to 50 r and 100 r were 63.1 per cent and 60.9 per cent, respectively, but these values were not significantly higher than that of the eggs from the control parents due to the wide variations within the groups. The mean percentage mortalities among the eggs obtained from parents exposed to 500 r, 750 r, 1000 r, and 1500 r were 84.8, 96.6, 98.3, and 99.8, respectively, all of which were significantly greater than that of the control.

Solberg (1938b) and others have shown that irradiation of embryos retards the rate of development. The following test was performed

to determine the effect of irradiation of the germ cells within the parent fish upon retardation of embryonic development. The number of temperature units required for development to hatching was calculated for each egg lot by a method described by Wallich (1901). The mean number of temperature units required for hatching the eggs from parents of each irradiation group was compared with the mean of the controls by the t-test (Table IV). Although the mean number of temperature units required to hatch the eggs from parents receiving a relatively high amount of X-rays was somewhat greater than that of the control group, significance could be demonstrated only for eggs obtained from parents receiving 1000 r. Because the mean numbers of temperature units required to hatch the eggs from fish exposed to 500 r, 750 r, 1000 r, and 1500 r were consistently higher than for eggs from control, 50 r or 100 r parents, the data were pooled into two groups of high or low exposure. The low exposure group required an average of 644.8 temperature units for hatching while the high exposure group required an average of 678.2 temperature units, the high exposure group thus required about five per cent more temperature units to reach the same stage of development. These means were compared by using the t-test and the difference between them was found to be highly significant.

Abnormalities

A majority of the eggs which died during the incubation period contained conspicuously abnormal embryos. In a strict sense, nearly all of the eggs failing to hatch were abnormal or else they would have survived; this discussion, however, will be confined to such teratoid embryos as could be easily recognized.

Some indication of the relative numbers and forms of the teratoid individuals can be gained from Figure 1 since the greater part of the mortalities in each stage of egg development was made up of such individuals. A concept of the polymorphic character of the abnormalities can best be had by the study of Figure 2, 3, 4, 5, 6, and 7, which are photographs of dead eggs. Most of the eggs showing no development were removed before the photographs were taken in order that a larger number of the teratoid forms might be included.

The deformities produced took on almost all conceivable forms except those of twinning, spina-bifida, cyclopians, and the like, which

TABLE IV

RELATIONSHIP BETWEEN TREATMENT OF PARENTS AND TEMPERATURE UNITS REQUIRED TO HATCH EGGS

Eggs from Control Parents				Eggs from Parents Exposed to 50 r				Eggs from Parents Exposed to 100 r				Eggs from Parents Exposed to 500 r			
Lot No.	Date Fertilized	Date Hatched	Temp. Units	Lot No.	Date Fertilized	Date Hatched	Temp. Units	Lot No.	Date Fertilized	Date Hatched	Temp. Units	Lot No.	Date Fertilized	Date Hatched	Temp. Units
1	2/6	3/26	622	5	2/8	3/29	643	6	2/10	3/31	649	17	2/23	4/10	653
2	2/6	3/27	637	25	3/12	4/21	627	16	2/23	4/14	719	18	3/3	4/21	742
3	2/6	3/27	637	27	3/12	4/21	627	20	3/8	4/19	644	26	3/12	4/25	699
9	2/10	3/31	649	29	3/12	4/21	627	23	3/12	4/23	663	28	3/12	4/25	699
12	2/14	4/4	662	34	3/15	4/25	659	24	3/12	4/23	663	30	3/15	4/25	659
22	3/9	4/20	649	44	3/19	4/28	662	37	3/15	4/25	659	38	3/15	4/25	659
32	3/15	4/25	659	47	3/21	4/26	600	40	3/17	4/24	616	41	3/17	4/28	688
33	3/15	4/25	659	51	3/23	4/30	649	58	3/26	5/2	645	56	3/26	5/5	701
59	3/26	5/4	682	67	4/9	5/9	558					66	4/7	5/9	607
	Mean $\bar{x}$		650.7		Mean $\bar{x}$		628.0		Mean $\bar{x}$		657.3		Mean $\bar{x}$		678.6
					t		1.84		t		.57		t		1.97

Eggs from Parents Exposed to 750 r				Eggs from Parents Exposed to 1000 r				Eggs from Parents Exposed to 1500 r			
Lot No.	Date Fertilized	Date Hatched	Temp. Units	Lot No.	Date Fertilized	Date Hatched	Temp. Units	Lot No.	Date Fertilized	Date Hatched	Temp. Units
39	3/15	4/24	641	8	2/10	4/4	714	7	2/10	4/6	747
43	3/19	4/25	608	10	2/10	4/4	714	54	3/26	5/2	645
46	3/21	4/27	618	15	2/19	4/9	683	55	3/26	5/3	663
49	3/23	5/2	688	21	3/8	4/21	679				
50	3/23	5/5	744	31	3/15	4/27	695				
52	3/23	5/5	744	35	3/15	4/26	677				
53	3/23	5/5	744	36	3/15	4/24	641				
57	3/26	5/3	663	48	3/21	4/27	618				
63	4/3	5/9	673	64	4/3	5/9	673				
65	4/3	5/8	646								
	Mean $\bar{x}$		676.9		Mean $\bar{x}$		677.1		Mean $\bar{x}$		685
			t 1.44				t 2.20[1]				t 1.78

Pooling

Eggs from Control Parents, from Parents Exposed to 50 r, and from Parents Exposed to 100 r

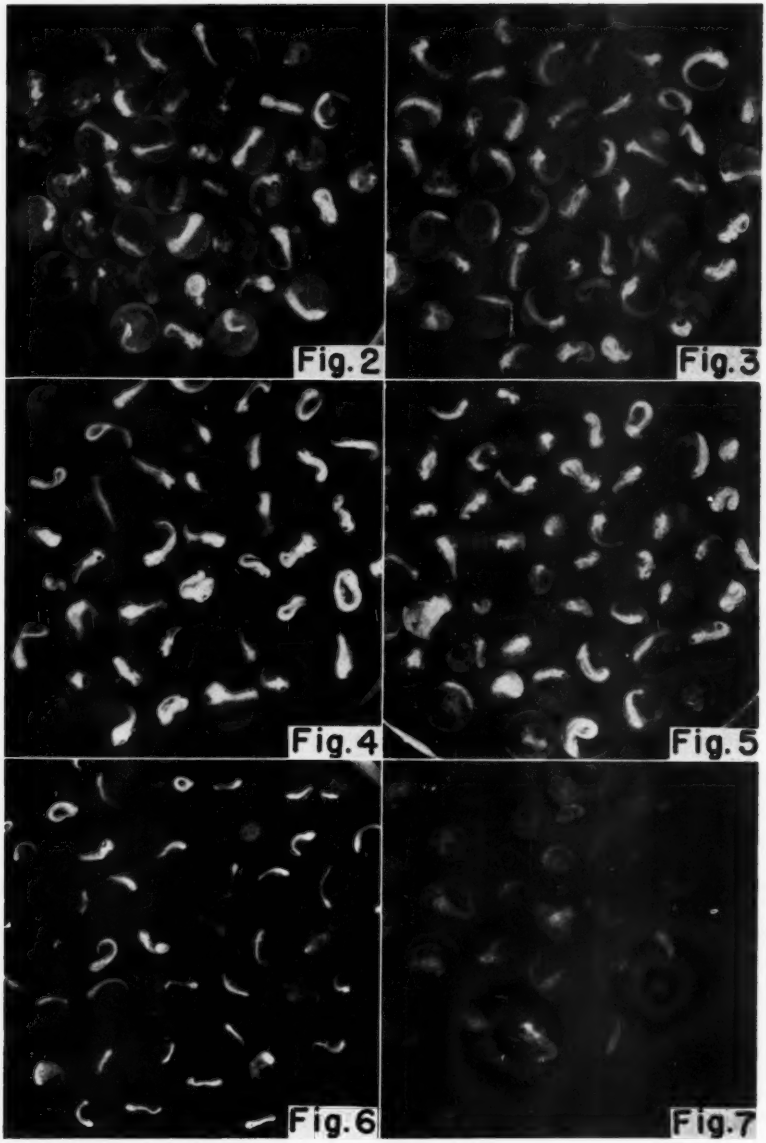
Mean no. of temp. units is 644.8

Eggs from Parents Exposed to 500 r, 750 r, 1000 r, and 1500 r

Mean no. of temp. units is 678.2

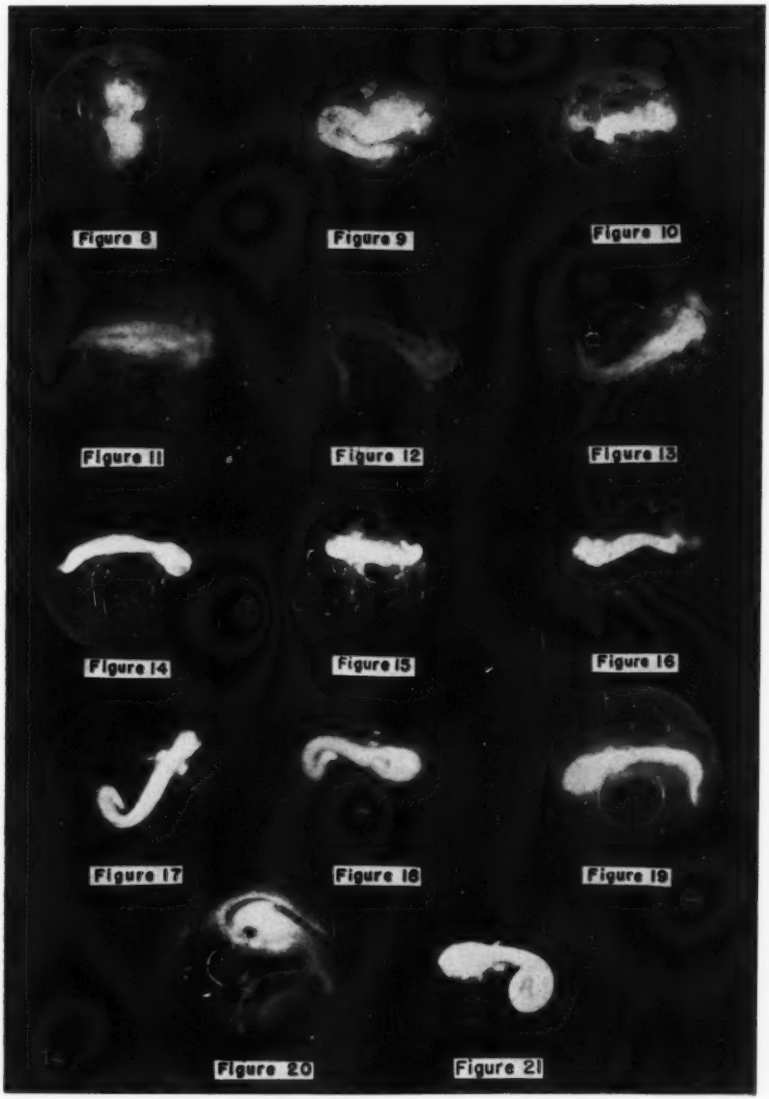
$t = 3.47$[2]

[1]Indicates a probability of less than 5 per cent that a greater number of temperature units were not required than in the controls.
[2]Indicates a probability of less than 1 per cent that a greater number of temperature units were not required in the high radiation levels than in the low radiation levels.



FIGURES 2, 3, 4, 5, 6, AND 7
PHOTOGRAPHS OF ABNORMAL TROUT EGGS FROM PARENTS EXPOSED TO X-RAYS. × 2.

Figure 2. Eggs from control parents.
Figure 3. Eggs from 100 r parents.
Figure 4. Eggs from 500 r parents.
Figure 5. Eggs from 1000 r parents.
Figure 6. Eggs from 1500 r parents.
Figure 7. Eggs from 2500 r parents.



FIGURES 8-21

PHOTOGRAPHS OF ABNORMAL RAINBOW TROUT EGGS WHICH DIED AFTER REACHING VARIOUS STAGES OF DEVELOPMENT

Figure 8. Developmental stage *B*. The parents received 2500 r.

Figures 9, 10, 11, 12, 13, 14, 15, and 16. Developmental stage *C*. The parent fish had been irradiated with 1000 r, 1500 r, 2500 r, 2500 r, 1000 r, 100 r, 100 r, and 500 r respectively.

Figures 17, 18, and 19. Developmental stage *D*. The parent fish had been irradiated with 500 r, 1500 r, and 750 r respectively.

Figures 20 and 21. Developmental stage *E*. The parent fish had been irradiated with 1000 r and 500 r respectively.

so commonly result from injuring the embryo in early stages of development (Stockard, 1921 and others). Rather, the abnormalities found here might be attributed either to deficiencies, improper differentiation of cell masses, disproportionate growth, or to combinations of these factors, indicating a profound injury affecting the factors which control organization and differentiation or the precursors of organs and systems. The results ranged from irregular and undifferentiated cell masses through crude embryonic shields and axes, abortive attempts at brain and tail formation, disproportionate and irregular development or absence of sense organs and pectoral fins, curled and otherwise distorted caudal regions, to the formation of puny and only slightly deficient individuals. Figures 8 through 21 are photographs of individual abnormal eggs in various developmental stages.

Abnormalities occurred in the eggs from control fish which were almost identical with the types found in eggs obtained from parent fish receiving large amounts of radiation (Figure 2). This indicated that no radically new types of abnormalities were produced by the irradiation of the germ cells but that the nature of the injury was such that the frequency of appearance of some types was substantially increased. Further, since a great variety of teratoid forms occurred among the offspring, the treatments applied to the adults were not specific for the production of any one type of deformity.

Many abnormal embryos were not sufficiently deformed to prevent their hatching and once out of the shell membrane they could be more easily segregated from normal fry. Most of the deformed fish were removed and classified (Figure 22 through 37 and Table V). Less than ten per cent of the young fish from control parents were abnormal. The percentage increased with the amount of radiation until no normal fish hatched among those produced by parents exposed to 1500 r.

Abnormalities resulting from incomplete development seemed to be more closely associated with the number of r units received by the parent fish than did those resulting from structural modifications. In the control group about twenty-nine per cent of the abnormal fry did not progress to the size or development of normal individuals. This percentage increased with the amount of radiation received by the parents and in the 1500 r group none of the resulting fry were fully developed (Table V). The degree of retardation of development also was related to irradiation.

TABLE V

NUMBERS AND TYPES OF ABNORMAL FRY SHOWN AS PERCENTAGES OF THE TOTAL NUMBER OF ABNORMAL FRY IN EACH TREATMENT GROUP

Number or r units received by parents	0	50	100	500	750	1000	1500
No. of abnormal specimens examined	498	462	562	187	84	165	8
Developmentally deficient							
D_1	3.4	.9	2.3	1.6	0	27.9	37.5
D_3	9.4	12.1	21.9	25.7	22.6	46.1	62.5
D_3	15.1	27.1	31.5	55.1	64.3	21.8	0
D_4	1.4	1.3	2.5	2.1	2.4	.6	0
Total	29.3	41.3	58.2	84.5	89.3	96.4	100
Weak body structure (W)	7.2	25.5	26.7	57.2	71.4	78.8	50.0
Extravasation (H)	24.5	32.5	46.3	51.3	63.1	35.2	50.0
Serous fluid (S)	22.1	33.1	41.5	55.6	70.2	31.5	25.0
Spinal Curvature							
C_1	0	2.6	2.7	5.3	5.9	34.5	25.0
C_2	8.4	1.5	6.2	3.2	4.8	3.0	12.5
C_3	11.6	10.2	8.9	3.2	8.3	7.9	12.5
C_4	52.0	42.6	32.9	14.4	17.9	14.5	0
Total	72.0	56.9	50.7	26.1	36.9	59.9	50.0
Shortened body							
L_1	.8	.4	.2	.5	1.2	0	0
L_2	1.6	2.4	1.6	2.7	3.6	.6	0
Total	2.4	2.8	1.8	3.2	4.8	.6	0
Distorted jaw (J)	1.4	3.5	12.5	16.6	7.1	11.5	0
Defective eyes							
E_1	0	0	.5	.5	1.2	5.4	0
E_2	.2	1.9	.4	1.1	3.6	6.1	0
E_3	.2	8.9	2.3	5.9	3.6	.6	0
Total	.4	10.8	3.2	7.5	8.4	12.1	0
Nodule on cranium (K)	0	1.1	4.3	3.7	5.9	2.4	0
Twinning	.2	.2	0	0	0	0	0

From Table V it would seem that the frequency of spinal curvature is inversely related to the amount of irradiation up to the 500 r level. This is not actually the case, however, since it is a peculiarity of the method of computing the percentages. In the control group for example about seventy-two per cent of the abnormal fry had spinal curvature but the majority of these were otherwise normally developed and thus not included elsewhere. A similar situation prevailed with

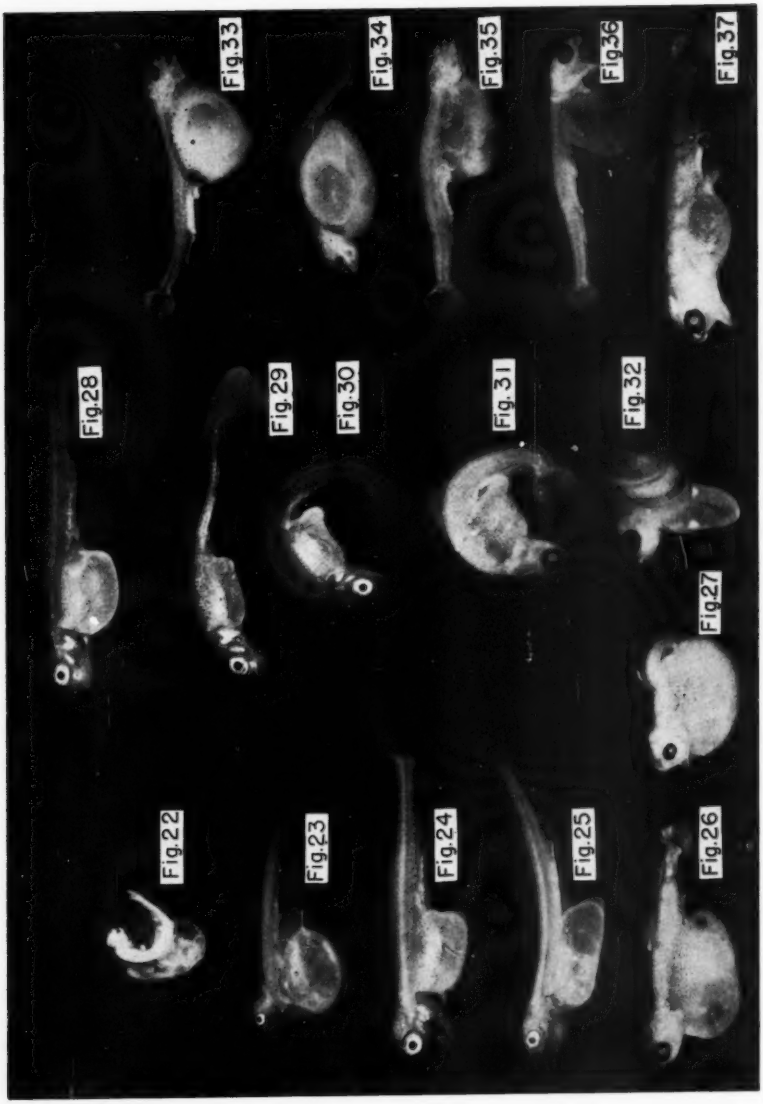


Fig.22
Fig.23
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Fig.34
Fig.35
Fig.36
Fig.37

FIGURES 22-37

PHOTOGRAPHS OF COMMON TYPES OF ABNORMAL FRY FOUND AMONG RAINBOW TROUT FRY

Figure 22. A normal rainbow trout fry which has developed to stage D_4.

Figure 23. A rainbow trout fry which typifies developmental stage D_1. Although hatched the embryo shows development more nearly characteristic of that found in the eyed stage.

Figure 24. A rainbow trout fry which typifies developmental stage D_3, and which has attained a size and development similar to that of normal embryos at the time of hatching. This specimen also has an accumulation of blood in the subocular sinus which is characteristic of abnormality classification H, and has a large amount of serous fluid in the extravitalline space which is characteristic of classification S.

Figure 25. A rainbow trout fry which typifies developmental stage D_3, approximately one-fourth of the yolk having been utilized.

Figure 26. A rainbow trout fry which is typical of classification W. The body and fins are weakly developed.

Figure 27. A rainbow trout fry with a distinct shortening of the body which is typical of classification L_2. Fusion of vertebrae has probably taken place.

Figure 28. A rainbow trout fry with a severely shortened body which is typical of classification L_1.

Figure 29. A rainbow trout fry with a slightly curved tail which characterizes classification C_1.

Figure 30. A rainbow trout fry with a moderately curved tail. Although this exemplifies classification C_2 the curvature is more severe than that found in most abnormal fry so classified.

Figure 31. A rainbow trout fry with a more severely curved tail which is typical of classification C_3.

Figure 32. A rainbow trout fry the tail of which forms a tight spiral. This typifies those classified as C_4.

Figure 33. A rainbow trout fry with subnormally developed eyes which is characteristic of classification E_2.

Figure 34. A rainbow trout fry in which the eyes are greatly reduced in size. This exemplifies classification E_2.

Figure 35. A rainbow trout fry in which both eyes are absent, typifying classification E_1.

Figure 36. A rainbow trout fry with a malformed lower jaw, a characteristic often associated with other deformities and classified as J.

Figure 37. A rainbow trout fry with a prominent nodule on the dorsal cranium. This illustrates classification K.

the 50 r, 100 r and 500 r groups. With the 750 r, 1000 r and 1500 r groups, on the other hand, many of the fry listed with spinal curvatures were also developmentally deficient or otherwise abnormal. The polymorphic nature of the abnormalities in the higher exposure groups is thus emphasized. Perhaps a better indication of the relationship between irradiation of parents and spinal curvature of embryos is seen in the change in relative proportion of slightly and severely curved fry. In the control group no fry were classified as having "very severe spinal curvature", but the number of such individuals increased with the amount of irradiation so that about half of the fry with curved spines in the 1000 r and 1500 r groups showed the "very severe" characteristics.

The abnormalities which occurred in the trout appear to have been much more extensive than those found by Solberg (1938a) for *Oryzias* where they were confined to deficiencies in size. The numbers and types of abnormal embryos produced in *Salmo gairdnerii* would seem to be more like those described by Corbella (1930) as a result of subjecting eggs of *S. irideus* and *S. lacustris* in cleavage stages to amounts of X-rays comparable to those used in this study.

Fry Mortality

The fry stage, which begins when the embryos hatch out of the egg and ends when the fish begin to feed actively, is affected less by interfering conditions than other stages of development. The mortality for this stage includes all fish which died between the time of hatching and May 26, 1945, and also all abnormalities which were removed during this time since the majority of these would probably have died very soon.

The direct relationship between mortality and amount of irradiation received by the parent fish was most clearly shown during the fry stage (Figure 38). The mortalities ranged from eight per cent in the controls to one hundred per cent in the 1500 r group. The variation in mortality between lots from similarly treated parents was considerably less in the fry stage than it was in the egg stage, consequently the increase in mortality with increase in dosage was more regular in the fry than in the eggs and the t-test had a greater sensitivity. The mean mortalities of the fry from irradiated parents, even those receiving only 50 r, were all significantly higher than the mean

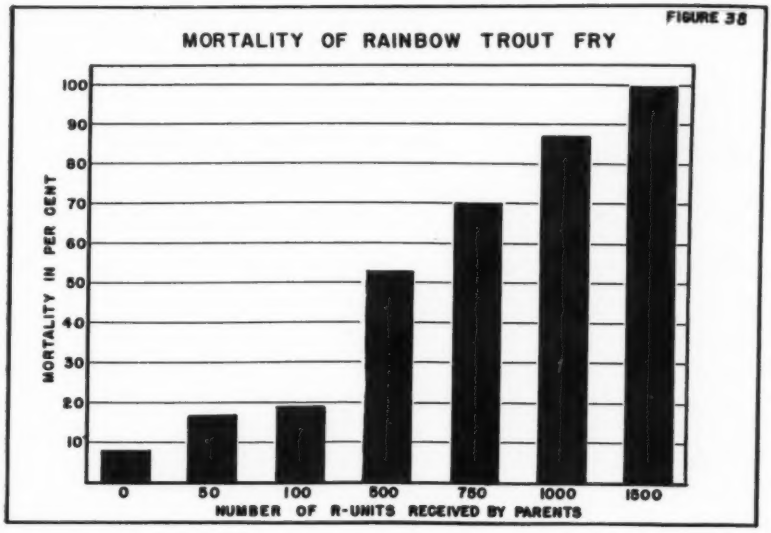

FIG. 38

mortality of fry of the control group. Values of t for irradiated groups compared to controls were as follows: 50 r, 2.94; 100 r, 3.46; 500 r, 8.44; 750 r, 23.14; 1000 r, 19.19; 1500 r, 14.44. Such a demonstrable difference at the low level of 50 r is of special interest.

Fingerling Mortality

After the young fish had started to feed growth was rapid and repeated reduction in the size of the lots or transfer to larger tanks was necessary in most cases to alleviate overcrowding. This section of the experiment was terminated on May 16, 1946, when the fish were about one year old.

As the size of the fish increased, mortality decreased and the differences in mortality rate between various groups became progressively less (Table VI). Significantly increased rates of mortality did exist, however, for as long as six months after hatching among offspring of parents which had received 500 or more r units. During the first year of life the mortalities among the fingerling fish ranged from 3.5 per cent in the control to 44.7 per cent in offspring of parents

TABLE VI

MORTALITY DURING THE FINGERLING STAGE

No or r Units Received by Parents	0 r		50 r		100 r		500 r		750 r		1000 r	
Date	No. Dying	Cum. %	No. Dying	Cum. %	No. Dying	Cum. %	No. Dying	Cum. %	No. Dying	Cum. %	No. Dying	Cum. %
5/26 to 6/26/45	(10132)		(5969)		(4194)		(794)		(131)		(38)	
	114	1.1	193	3.2	174	4.1	94	11.8	31	23.7	13	34.2
6/26 to 7/31/45	(489)		(489)		(473)		(458)		(92)		(25)	
	—				2	4.5	16	14.9	6	28.7	1	36.8
8/1 to 8/31/45	2	1.5	3	3.8	5	5.5	4	15.7	—		1	39.5
9/1 to 9/30/45	—		—		—		1	15.9	—		1	42.1
10/1 to 10/31/45	1	1.7	5	4.8	1	5.7	11	18.0	1	29.5	1	44.7
11/1 to 12/29/45	6	2.9	15	7.8	3	6.3	30	23.8	7	35.3	—	
11/30 to 12/31/45	(302)		(296)		(299)		(292)		(69)		(21)	
	2	3.5	2	8.4	1	6.6	4	24.8	1	36.1	—	
1/1 to 1/31/46	—		—		2	7.2	3	25.6	—		—	
2/1 to 2/28/46	—		—		—		—		—		—	
3/1 to 3/25/46	—		—		1	7.5	1	25.9	—		—	
3/26 to 5/16/46	(200)		(200)		(202)		(200)		(68)		(21)	
	—	3.5	—	8.4	—	7.5	1	26.3*	—	36.1*	—	44.7*

Parenthetical values (489) indicate number of live fish remaining in lots following reductions to alleviate overcrowding. Percentage mortalities marked * indicate differences from the control which were highly significant.

receiving 1000 r units. None of the embryos produced by parents irradiated with 1500 or more r units survived to the fingerling stage.

The cause of the continuing higher rate of mortality among the fingerlings of the higher dosage groups was not apparent from superficial inspection and so, erythrocyte counts were made. Peculiarly enough the lowest average count, 96.9 cells per 0.0001 cubic millimeter, occurred among the controls and the highest average count, 130.8 cells per 0.0001 cubic millimeter, occurred in the 100 r group. The inconsistent manner in which low and high average counts were arranged in these and other groups would seem to cast considerable doubt on any close relationship between erythrocyte count in fingerling fish and X-radiation of their parents.

Growth

The increase in size of the fish was followed by weight. The various groups of fish were first weighed on May 31, 1945, about the time they were first starting to feed, and subsequently they were weighed every second week until this section of the experiment was terminated in May 1946. At first, the fish were weighed in groups of one hundred but as their size increased the number weighed in each sample was reduced to fifty and then to twenty-five to facilitate handling. At the end of the experiment one hundred fish from each group, or all that remained alive in the 750 r and 1000 r groups, were weighed and measured individually so that a t-test could be run to determine the significance of the differences in the average size of the fish.

During the summer of 1945, the rate of growth was much the same in all of the groups with a tendency toward slower growth among offspring from parents treated with 500 r, 750 r, or 1000 r (Figure 39). After the middle of October, growth was greatly retarded and became somewhat irregular in all of the groups due to the low temperature of the water. During the poor growing conditions of the winter, growth of the fish in the high dosage levels was retarded appreciably more than that of the controls or the 50 r group, causing a gradual divergence of the curves. The 100 r group tended to be intermediate. Rapid growth was resumed about the first of March 1946, and continued to the end of the experiment. The inhibited growth rate of the groups receiving higher irradiations became even more apparent during the spring of 1946, and early in May the differences in the average size of the fish in the various groups were considerable. The

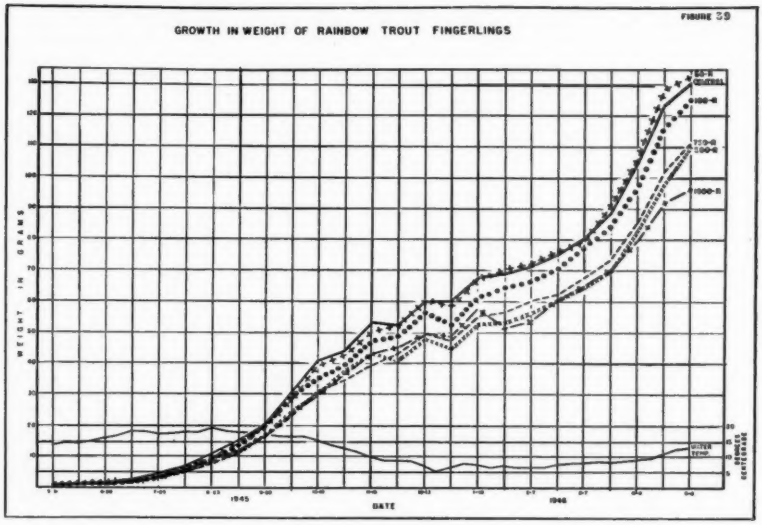

FIG. 39

size of the fish, and thus growth, were, in general, inversely related to the amount of irradiation received by the parent fish.

The 50 r and control groups had almost identical growth patterns and they were not statistically different. The mean weight of the 100 r group was consistently less than that of the control throughout the year and statistical tests confirmed that growth was slightly impeded at this irradiation level. Parents treated with 500 or more r units produced a progeny which grew appreciably more slowly than normal; statistically the difference was highly significant. At the end of the first year the mean weight of offspring from parents exposed to 1000 r was only seventy-one per cent of the expected weight.

Summary

One hundred and forty-eight rainbow trout (*Salmo gairdnerii* Richardson) approximately twenty months old and approaching sexual maturity were divided into a control group and seven irradiated groups. Total body irradiation doses of 50 r, 100 r, 500 r, 750 r,

1000 r, 1500 r and 2500 r were administered at a rate of 8.25 r per minute.

When the fish became mature about three months after irradiation they were spawned, and a total of 115,454 eggs were obtained from 67 females. Although there was an interval of sixty-two days between the time of spawning of the first and last female the data did not show that the X-ray treatments had either delayed or hastened spawning. Some of the fish exposed to 2500 r were so extensively damaged that they died before spawning. Since the parent trout were partially mature at the time they were X-rayed, their developing germ cells also received the radiation. The data did not indicate that germ cells injured by radiation tended to recover before syngamy.

The mean mortalities of the eggs obtained from parents subjected to 500 or more r units were significantly greater than that of the eggs from the control parents. Most of the eggs which died during the incubation period contained conspicuously abnormal embryos. The abnormalities could be attributed to deficiencies, improper differentiation of cell masses, disproportionate growth, or combinations of these factors. The deformities which occurred were of almost all conceivable forms except those of twinning, spina-bifida, cyclopians, and the like. Abnormal types of embryos occurred among the progeny of control parents and of parents which had received low doses of radiation which were almost identical with the types which occurred among the progeny of parents receiving large amounts of radiation. However, as the amount of radiation increased the relative abundance of malformed embryos increased and the degree of development attained decreased. Practically all of the embryos from parents treated with 1500 r and 2500 r were so abnormal that they died before closure of the blastopore. Irradiation of the parent fish thus increased the frequency of occurrence of malformations. Certain types of abnormalities seemed to be more closely associated with irradiation than did others.

During the fry stage of development the mortality in every group from irradiated parents, even those receiving only 50 r, was significantly higher than the mortality in the control group. After the young fish had started to feed the differences in mortality between the various groups became progressively less. Significantly increased rates of mortality did exist for as long as six months after hatching in groups receiving 500 or more r units.

The rate of growth of the young trout during their first year of life was also found to be directly affected by the amount of irradiation received by the parent fish. While variations in mortality became less with increasing age of the fish, variations in size became greater. Parents treated with 100 r produced progeny in which growth was slightly impeded, while parents treated with 500 or more r units produced progeny which grew appreciably more slowly than normal.

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BOOK REVIEW

Advances in Genetics, Volume 2, M. Demeric, Editor. Academic Press, Inc., New York, N. Y. 373 pp.

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The present volume of the *Advances in Genetics* continues the attempt at synthesis within the field, implicit in the concept of the series. Such projects are always difficult. The difficulties are particularly evident at the present time in Genetics, where the centrifugal forces tending to separate off different sub-branches of the field are so strong. But precisely for this reason, the series has the value of placing under the same cover a group of papers which cover such apparently unrelated topics as the cytology of the cocids and cytoplasmic inheritance, thus forcing their consideration as parts of a single discipline.

The volume contains seven articles, ranging in length from the brief monographs of Caspari, Hughes-Schrader and Catcheside, to shorter reviews by Heston, Dahlberg, Mayr and Sears. The range in subject matter is even wider, encompassing a broad discussion of the nature of species and a detailed treatment of the genetic effects of radiation.

Genetics may be considered as the study of the mode of inheritance of individual traits, thus a way of finding out about the characteristics of individuals or populations. Or—and this is the point of view at the root of the current growth of chemical genetics—it is an effort to understand the nature of the hereditary units. Both these approaches are exemplified in the volume.

Of particular value is the review of cytoplasmic inheritance, by Caspari. Much of the older literature, especially the theoretical discussion in the botanical papers, is not as familiar as it should be. Caspari comes to the conclusion that "the number of cases described, though not large, is now large enough to suggest that a normal

phenomenon is actually involved which is not as easily detected as mendelian inheritance". The difficulty with this approach, however, is one not generally recognized: the proof of cytoplasmic inheritance offered is usually a negative one. These are cases in which the inheritance cannot be explained according to an orthodox chromosome analysis; but the random segregation of chromosomes to give mendelian results is not invariable. The caution in interpreting curious cases of inheritance as necessarily extra-chromosomal without a detailed cytological understanding of the material, is reinforced by an almost neighboring review, that of the cytology of the coccids by Hughes-Schrader. If the genetics were known, and not the cytology, in such an organism as Pseudococcus, where one whole set of chromosomes never appears in the sperm, the behavior of a dominant in the female line would be quite unorthodox, certainly non-mendelian. Regular departures from the random segregation of the chromosomes must be considered before the existence of autonomously reproducing bodies in the cytoplasm can be demonstrated.

The case of the kappa factor in Paramecium or of the plastid inheritance in various plants, in which interrelations have been found between genes and cytoplasmic factors, gave rise to a theory of "plasmon" sensitive genes, genes whose phenotypic effects were determined by the nature of the cytoplasm in which they found themselves. From this it is a step to the presentation of Spiegelman, in which the concept of a plasmon, vague in the earlier work, is made diagrammatic, is formulated as a system of competing chemical reactions. Since Caspari's review was written, the climate of opinion has changed markedly; geneticists are now (with some exceptions) beginning to feel the hangover after an orgy of extrapolation from data. The best accredited cases of cytoplasmic inheritance turn out to be studies of symbiosis or parasitism, with the cytoplasmic factors either helpful to the host (plastids) or indifferent, but producing side effects by which their presence is detected (kappa). Definitive evidence of a system of autonomously replicating units in the cytoplasm has yet to be presented.

The prompt acceptance of these cases as fundamental to concepts of gene action deserves comment: so strong is the desire, and also so great the necessity, to use the techniques of genetics (crossing experiments of various types) for the study of gene action, that the

cases in which effects were transmitted through what appeared to be cytoplasm, were immediately interpreted as providing a direct insight into the workings of the genes. This, it would now appear, is not the case: we are back in position, with the concepts of the interaction of genes and cytoplasm not notably advanced over the earlier work, for example, of Hämmerling on Acetabularia.

The discussion turns also to the relation between viruses and plasmagenes. The argument that viruses and plasmagenes are arbitrarily defined, and are really not distinguishable, would seem to beg the question, whether the mode of action of the nucleus of the cell is to set up a lesser hierarchy of autonomously reproducing bodies, or to act in some other way. The assumption that a normal constituent of one type of cell may parasitize another, thus becoming a virus by definition, leaves the nature of the normal function and relationships of the constituent still undefined.

A further comment on the theoretical discussion of Caspari's review: his major thesis is that the facts of cytoplasmic inheritance can be explained by a system of chemical reactions not unlike that postulated by Stern for the position effect. The logic of this argument is rather difficult to follow. Clearly, the system of competitive chemical reactions postulated are common to all biological processes, and cannot furnish a distinction between one type of mechanism or another.

The studies of the Schraders on the coccids are already classical. The standard techniques of cytology consist in the close comparative study of a group of organisms, the idea being that the variations within the group furnish a series of "natural experiments", which can be analyzed to provide an understanding of the variables in the case. This type of approach is now used for the study of the physiology of the chromosomes by the Schraders, in their organisms; certainly the results give evidence of the effectiveness of the technique, in posing problems for experiment. Equally, the necessity of experiment is indicated, in order to avoid an otherwise unfortunate tendency in cytology towards circular argument, in which each attraction is counterbalanced by its partner repulsion, and differences in timing supply whatever other arbitrary constants are needed to make a good story. Oddly enough the method has its successes; the stage of mitosis were worked out in this way, and in the present review, the inference

of the diffuse kinetochore, deduced by Schrader from the configurations of the chromosomes, was proved to be correct by the x-ray experiments of Ris and Hughes-Schrader. It is instructive, in this regard, to observe how comprehensive a view of the organism is necessary; the taxonomy of the coccids, their life cycles, their embryology, all are necessary to a proper study of the chromosomes; and as is pointed out in a final paragraph the material is useful for the study of other untouched problems in cytoplasmic cytology, and in the study of host parasite relations. Notable features of the chromosome study in this material, aside from the diffuse kinetochore, are the demonstration of multiple strand structure of the chromosomes, the tendency of the individual chromosomes to form their own nuclear vesicles and the analysis of chromosome orientation in the sperm. The work as a whole provides a much needed corrective to a standardized approach to chromosome physiology, although it perhaps suffers from an understandable tendency to delight in the exotic for its own sake.

Three of the reviews form a group, concerned with the genetic properties of species and populations. The general paper by Ernst Mayr, on the Nature of Species, is an attempt to marshal the steadily increasing body of data in order to clarify a semantic problem. Before Darwin, each new species was a discovery; since the publication of the "Origin of Species" each has become a new problem in genetics. The definition of species which Mayr and his co-believers would like to set up has its basis in the study of the breeding structure of the populations. A species is a population which is reproductively isolated from its closest relatives, in such a way that the change in its genetic structure resulting from new gene mutation is as great as any resulting from hybridization with members of other populations. Visible differences between two such reproductively isolated populations are not required to exalt them to the status of species—a point of view which seems to have caused acute discomfort to workers who believe that one should be able to distinguish the individual members of different species by looking at them. The difficulty seems largely to be one of the overtones of meaning; having, in its older incarnation, the implication of a discovery, the term species has an aura which in the point of view supported by Mayr, is extended to the idea that a species is a "fact of nature" and not simply a convenience of

the taxonomist. He rejects the term physiological race, which had been in progress of becoming a term used for reproductively isolated populations morphologically undistinguishable. The discussion is a most interesting example of the changes in usage of words as the techniques of the disciplines change: the wines of the new systematics are poured into the bottles of an old species terminology.

The two other papers, one by Sears on the cytogenetics of the wheats, and the other by Dahlberg on the genetics of human populations, are examples of the different types of individual analyses that must accumulate to give body to general discussions of species. The intricacies of Triticum genetics are concisely reviewed here. Sears himself has notably contributed to their understanding; of particular interest is the identification of the different genetic chromosomes in these polyploid species by the analysis of the effects of complete absence of a particular pair of chromosomes.

In Dahlberg's review, the establishment of a basic human genetics is taken for granted: particular linkages, the modes of inheritance of characters in man are not discussed, being the preliminary to theoretical analyses by mathematical techniques of the changes in the genetic structure of populations under different systems of mating. The formation of isolates, populations of distinctive genetic structure is a key problem, here as elsewhere in population analyses. The possibilities examined, he points out in an instructive section their relevance to theories of the genetic structure of the classes of a society: "Even if the upper classes resulted from selection, there is naturally no reason why we should be satisfied with the effect achieved. If an upper class of particularly high quality is wanted, the simplest thing should be to give everyone the same chance of working up to a leading position. Genetically speaking, this would mean a gain, since the selection would then have a wider scope than if it were made only from a smaller part of the population." The analysis at present is at a primitive stage. "We require both knowledge of the frequency of intermarriage, assortative mating, the formation of isolates, etc., and also investigations of the actual frequency of individual characters in populations.—A great deal must be done to achieve an empirical foundation for the assessment of populations from the viewpoint of heredity."

The two remaining papers of the volume are of direct interest to

students of growth. The longer of the two is a pocket monograph by Catcheside on the genetic effects of radiation. In seventy-eight pages, the attempt is made to cover a field ranging from the presentation of the physical characteristics of the radiations, the cytologically observed effects on chromosomes, analyzed with respect to the mitotic cycle, and the wide variety of genetical analyses on Drosophila and maize, for example. In spite of the fact that the references number 381, "the survey is necessarily selective and much literature of little analytical value to our purpose"—the nature of the genetic changes and of the gene itself—"is neglected." The field has often been reviewed; the usefulness of any particular treatment, even if it be not altogether novel, resides in the attempt to integrate the more recent advances with the older literature. From this point of view, one would have preferred possibly omission of some chapters, with a less condensed analysis of the various factors determining chromosome breakage and reunion. The conclusion reached is that "no theory accounts satisfactorily for all the facts and that both the physical and physiological hypotheses have a value in providing further experiment." It is evident that the whole problem of the nature of radiation effects, now increasingly of practical as well as theoretical interest, is beginning to approach a stage in which restatement will be necessary, in less inclusive but more detailed terms. The data reviewed represent the most refined quantitative analysis of radiation effects on a biological system, and the methodology, particularly in the application of the target hypothesis, deserves close attention.

Finally the short review by Heston of the Genetics of Cancer, gives a short resumé of the present status of one of the major growth problems. Heston approaches the problem from the point of view of the susceptibility to cancer as "a physiologic character involving growth processes". The studies of the genetics of cancer have been carried out chiefly in the mouse, thanks to the establishment of inbred lines varying in their susceptibility. The over-all conclusion reached from these studies places the character of cancer formation in the same category as other growth changes: determined by many diverse genes, different ones being of importance in different types of cancer. Among the more interesting of the special cases is the analysis of the genetic influences surrounding the milk factor (virus)

for the formation of mammary tumors. Two sets of factors seem involved, those governing the propagation of the factor and the susceptibility to it. One is caught up by a feeling of surprise however, that in such a review there is no mention of the outstanding problem in malignancy, the genetic nature of the cancer cell itself. Only in one place, and that a discussion of the already over-discussed plasmagene, is there even an oblique mention of the problems of cellular genetics in cancer. It is true that any discussion at the present time is bound to be largely speculative; yet a discussion of the genetics of cancer which ignores questions of somatic mutation, and the nature of the factors influencing it, must be considered incomplete.